— SECTION B —

CHEMICAL SCIENCES

Contents

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NOTICE

The Canadian Journal of Research is at present published in six sections, A to F. Starting with January 1, 1951, these sections will be published as separate journals under distinctive names and the designation Canadian Journal of Research will no longer be used. The present names and the corresponding new names are as follows:

Present Name	*New Name*
Canadian Journal of Research, Section A (Physical Sciences)	Canadian Journal of Physics
Canadian Journal of Research, Section B (Chemical Sciences)	Canadian Journal of Chemistry
Canadian Journal of Research, Section C (Botanical Sciences)	Canadian Journal of Botany
Canadian Journal of Research, Section D (Zoological Sciences)	Canadian Journal of Zoology
Canadian Journal of Research, Section E (Medical Sciences)	Canadian Journal of Medical Sciences
Canadian Journal of Research, Section F (Technological Sciences)	Canadian Journal of Technology

In order to preserve continuity the present sequence of volume numbers will be retained, and in each case the volume for 1951 will be Volume 29.

The subscription rates for the Journals will remain as at present.

ESTIMATION OF REDUCING SUGARS IN STARCH HYDROLYZATES BY PAPER CHROMATOGRAPHY[1]

BY PING SHU

Abstract

A method for quantitative determination of glucose, maltose, and reducing dextrins in starch hydrolyzates is described. The components are separated by paper chromatography and determined colorimetrically by measurement of their reducing power with alkaline copper and arseno-molybdate reagents. The probable error of the determinations with the use of this method is about 2%. A compact apparatus was designed, suitable for handling a large number of samples in the chromatographic separation. It is particularly useful for compounds with low R_f values.

Introduction

The extent of starch saccharification is commonly estimated by determining the increment of reducing power of the hydrolyzate by the use of alkaline ferri-cyanide or copper reagents. Because of the accumulation of reducing dextrins, this increment of reducing power may not be altogether accounted for by the formation of reducing sugars. By a method of differential fermentation with yeast, Somogyi (15) and Schultz and Kirby (14) were able to determine the composition of sugar mixtures.

In recent years, paper chromatography has been widely used for the resolution of mixtures of compounds. The invention of devices for holding paper strips enhanced the application of ascending paper chromatography (2). The use of a filter paper cylinder or concentric cylinders provides the simplest method of handling the paper (17, 18). The introduction of the center-tailed paper disk (13) and the confined channel (3, 4) techniques greatly shortened the development time. Miettinen and Virtanen reported a "continuous developing technique" in descending paper chromatography by attaching a thick pad of cellulose material at the foot of the paper strip (9).

A series of articles on the development of quantitative chromatographic methods for sugar analysis have been reported by Hirst and coworkers (5, 6, 7). Combining the techniques of differential fermentation and paper chromatography, Portor and Fenske were able to analyze a mixture of glucose, galactose, and rhamnose (12). However, none of these methods are suitable for the handling of a large number of samples.

The purpose of this report is to describe a method of determining glucose, maltose, and reducing dextrins in starch hydrolyzates. The method, which is suitable for manipulating a large number of samples, consists essentially of the separation of the sugars and reducing dextrins by paper chromatography, and the determination of the reducing power of each separate band by an alkaline copper reagent.

Experimental

Reagents

Alkaline copper reagent (16).—One liter aqueous solution contains 28 gm. of anhydrous disodium phosphate, 200 ml. of N sodium hydroxide, 40 gm. of Rochelle salt, 8 gm. of copper sulphate pentahydrate and 180 gm. of anhydrous sodium sulphate.

Arseno-molybdate reagent (10).—Dissolve 25 gm. of ammonium molybdate in 450 ml. distilled water, then add 21 ml. concentrated sulphuric acid and 0.5 gm. of "Duponal C".* Add 3 gm. disodium arsenate heptahydrate dissolved in 25 ml. water. Mix and make up to 500 ml. volume with distilled water. Incubate at 37°C. for 24 to 48 hr.

Seventy-five per cent saturated sodium sulphate solution.—Dilute 75 ml. of saturated sodium sulphate solution to 100 ml. with distilled water.

Color indicator for sugars (11).—Dissolve 1.66 gm. of phthalic acid and 0.93 gm. of redistilled aniline in 100 ml. water saturated butanol.

Developing solvent (8).—Mix ethyl acetate, acetic acid, and water in the proportion 3:1:3 (by volume) and use the top layer.

Apparatus

A device for holding a large number of paper strips was designed. As shown in Fig. 1, it consists of a rectangular glass (or stainless steel) stand 5 in. high, with a cross section of 6 in. by 6 in. Five filter paper pads are placed side by side in rows on the top of the stand. Each pad is made of 40 layers of 1 in. by 6 in. Whatman No. 1 paper strips. These strips are held between two 1 in. by 6 in. stainless steel plates, which are bolted together. At the bottom of the stand, there are six evenly spaced parallel cross bars, including the two frames of the stand. A rectangular plate glass, slightly narrower than the cross section of the stand, is laid under the cross bars. This plate is so supported that it may slide in and out of the stand in a direction at right angles to the parallel cross bars.

The upper ends of the chromatographic paper strips (1 in. by 7.5 in. Whatman No. 1 filter paper) are evenly attached to the pads. The lower ends of the strips are held between cross bars and the glass plate at the bottom of the stand. This may easily be arranged by sliding the glass plate in position when the rows of the chromatographic paper strips are hanging downwards from the pads and in front of the cross bars. Since five paper strips may be attached to each pad, the stand described above holds 25 strips, each 1 in. wide.

* *A product of the E. I. du Pont de Nemours and Co. (Inc.), Wilmington, Delaware.*
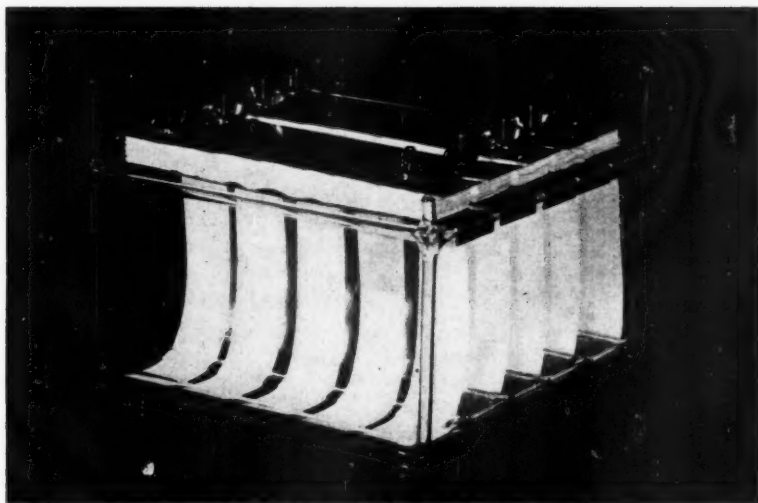
FIG. 1.   *Assembled paper strip stand.*

Insertion of the paper strips into the pad is greatly facilitated by use of a holder. This holder contains five compartments into which the paper strips are inserted. It is made by cementing six parallel celluloid strips 1/8 in. thick between two celluloid sheets (each 6 in. by 6 in. by 1/16 in.). One of the ends is closed by another strip of celluloid. When the paper strips are put in the compartments they are held parallel. The exposed ends project enough to be readily attached to the pad. This is accomplished by laying them in the middle of the layers of pad papers and tightening the nuts. The holder is then removed. Its use is illustrated in Fig. 2.
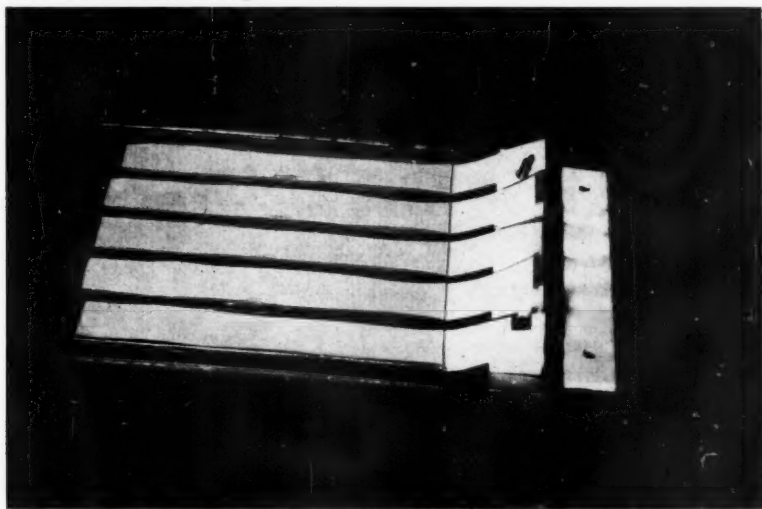
FIG. 2.   *Attachment of paper strips to the pad with the aid of a holder.*

Procedure

After the paper strips have been attached to the pads with the aid of the holder, a straight pencil line is drawn across the strips about 4.5 in. from the pad. An accurately measured amount of sample (about 0.01 ml.) is applied to each strip by streaking with a capillary pipette along the pencil line. The band width of the applied streak should be kept about 1/8 in. by drying over a gentle stream of air during application. The air current may be conveniently obtained by connecting a fin-tipped short stem Bunsen burner to an air line. Samples are drawn into the pipette either by capillary rise or by suction. After filling, the solution adhering to the outside of the pipette tip should be washed off and the tip then dried by wiping with a piece of soft paper. The liquid level in the pipette may easily be adjusted by touching the pipette tip to a piece of filter paper.

After the application of samples to the strips, the pads are placed on the stand and the lower end of the strips are held to the bottom of the stand between the glass plate and the cross bars, as described in the previous section. The loaded stand is then enclosed in a glass chamber containing developing solvent. The solvent level is so adjusted that the sample lines are about 1/2 in. above the surface of the liquid. A satisfactory separation may be obtained in a period of 9 to 12 hr. The development should not be longer than 17 hr., or the glucose band will enter the pad. The developed strips are then air dried, and detached from the pad. An indicator strip is sprayed with the phthalic acid – aniline – butanol reagent and heated at 100°C. for five minutes. Glucose and maltose appear as two distinct brown colored bands. Reducing dextrins do not move appreciably in this developing solution. The position of the bands on the remaining strips are located, using the indicator strip for reference. The bands are then cut out and put into separate clean test tubes. Since the upper edge of the band generally tends to diffuse at the side of the strip, a section from each side about 3/8 in. high and 1/4 in. wide is included in the sample. The reducing values of the bands are determined on their extracts when the band occupies an area larger than 1/4 sq. in.; otherwise the reducing values can be estimated in the presence of the filter paper.

In general, for glucose bands, the determinations are made on their extracts. Here, 1 ml. of sodium sulphate solution is added to the sample (all the filter paper should be immersed), and this is heated in a boiling water bath for five minutes. After cooling in running water, an 0.5 ml. aliquot of the extract is removed for analysis. With maltose and reducing dextrin determinations, 0.5 ml. of the sodium sulphate solution may be directly added to the test samples without the removal of filter paper.

To each of these tubes, 0.5 ml. of alkaline copper reagent is added with thorough mixing, and the tubes are then heated in a boiling water bath for 20 mins. Immediately after cooling to room temperature, 0.5 ml. of arseno-molybdate reagent is added to each tube. When the color is fully developed (about five minutes), 4.5 ml. of distilled water is pipetted into each sample.

(The reducing dextrins and maltose samples are allowed to stand for 30 to 60 min. to secure an equilibrium distribution of molybdenum blue between filter paper and the solution.) Five milliliter aliquots are removed and diluted to 15 ml. with distilled water. The optical density of the diluted solutions is determined using the Evelyn colorimeter, with a 620 mμ filter. If the samples exhibit a reducing value greater than that for 100γ of glucose, a further dilution should be made. Paper blanks and sugar standards must be included.

After washing and drying, the paper pad can be used over again.

Results and Discussion

The use of a pad and stand is not only convenient for holding a large number of samples, but also hastens the separation process. In ordinary ascending paper chromatography, because of the reduction of the capillary head, the ascending rate of the solvent front decreases as it ascends. However, with the pad fixed at a short distance from the surface of the liquid, the movement of the solvent is kept constant at a high rate. Some comparative results are given in Fig. 3. The present device is particularly suitable for handling mixtures containing compounds of low R_f values.
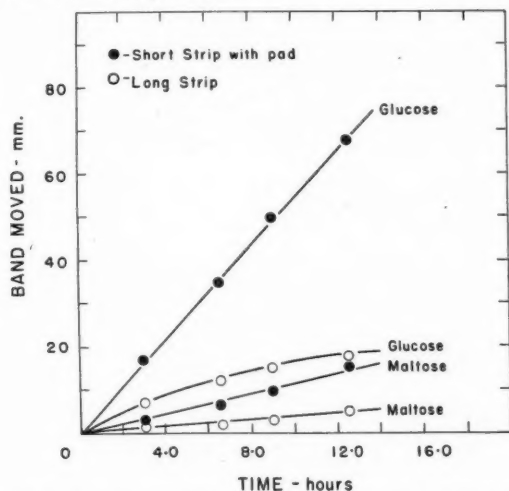
Fig. 3. *Rate of chromatographic development on long and short paper strips.*

During the development of this method, it was found that the direct use of Somogyi's (16) and Nelson's (10) reagents in the present method is not possible. The difficulties are: (*a*) poor reproducibility of the results at the low range of reducing value (5–100 γ glucose), mainly caused by the reoxidation of cuprous oxide; (*b*) the loss of alkalinity of the copper reagent by the introduction of acetic acid which has been carried over with paper strips; (*c*) molybdenum blue tends to adsorb on paper when the determination of reducing value is made in the presence of filter paper.

The reoxidation of cuprous oxide is prevented by using 75% saturated sodium sulphate solution in the preparation of the test samples. The effect of the acetic acid carried over with the paper can be eliminated by doubling the sodium hydroxide concentration in the Somogyi's alkaline copper reagent without affecting the sensitivity of determination. The use of "Duponal C" hastens the uniform distribution of molybdenum blue between paper and solution. The "Duponal C" itself does not interfere with the color reaction.

In a preliminary experiment, it was found that the filter paper alone will reduce the alkaline copper reagent to a certain extent. The reducing power of the filter paper is not changed by prolonged extraction with boiling water and is remarkably uniform. The analysis of 10 samples taken from different sheets of Whatman No. 1 filter paper shows that a 1/2 sq. in. size gives a reducing value equivalent to 20.1 γ glucose with a standard deviation of 0.61 γ.

In order to test the reproducibility and the over-all recovery by this method, determinations were conducted on three samples containing varied amounts of glucose and maltose. Six or seven replicate determinations were made on each sample. The data summarized in Table I show that higher recovery may

TABLE I

TESTS ON PRECISION AND RECOVERY

Paper strip No.	Glucose		Maltose	
	Added, γ	Recovered, γ	Added, γ	Recovered, γ
1	65.0	62.0	24.8	24.8
2	65.0	61.0	24.8	24.8
3	65.0	63.0	24.8	22.0
4	65.0	62.0	24.8	22.0
5	65.0	63.0	24.8	23.0
6	65.0	58.0	24.8	23.0
	Average recovery: 94.6%		Average recovery: 93.9%	
	Probable error: 1.9%		Probable error: 3.1%	
7	194	182	69.0	72.0
8	194	194	69.0	72.0
9	194	194	69.0	70.5
10	194	190	69.0	72.5
11	194	192	69.0	70.5
12	194	194	69.0	74.5
	Average recovery: 98.5%		Average recovery: 104%	
	Probable error: 1.5%		Probable error: 1.3%	
13	134	130	93.5	91.5
14	134	131	93.5	93.5
15	134	128	93.5	90.5
16	134	134	93.5	93.5
17	134	132	93.5	87.0
18	134	132	93.5	87.0
19	134	126	93.5	86.0
	Average recovery: 97.3%		Average recovery: 96.1%	
	Probable error: 1.3%		Probable error: 2.2%	
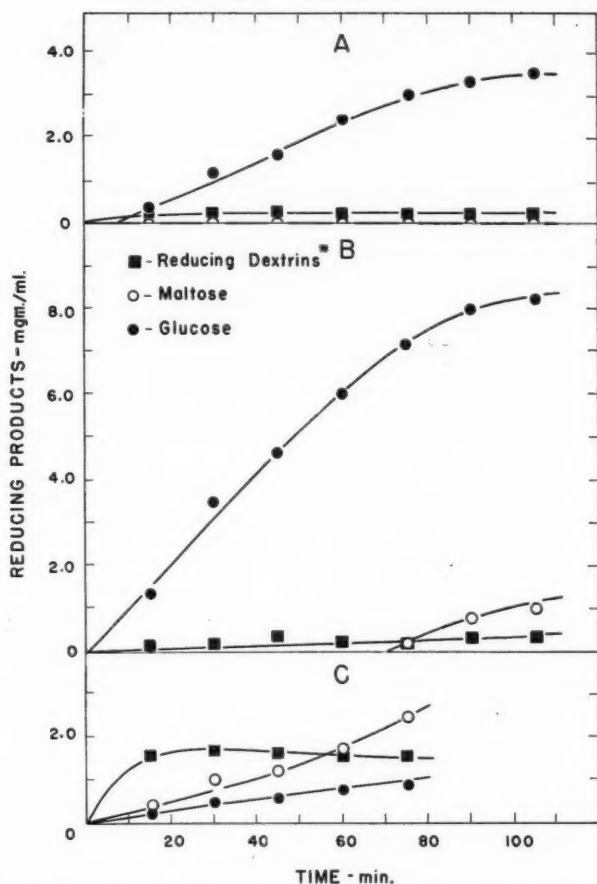
FIG. 4. *Hydrolysis of starch by enzyme preparations having various maltase and amylase activities.*

> A. *20.4 units of maltase activity and 76 units of amylase activity.*
> B. *46 units of maltase activity and 298 units of amylase activity.*
> C. *1350 units of amylase activity and a trace of maltase activity.*

be obtained at higher sugar concentrations. Within the present experimental range, minimum recovery is about 94%. The probable error of the determination is about 2%. Thus, the precision of this method seems to be satisfactory for general experimental use.

To demonstrate the applicability of this method for following the enzymatic hydrolysis of starch, the time versus hydrolysis relationship for enzyme preparations from commercial α-amylase and *A. niger* culture having various maltase and amylase activities† was investigated. Five milliliters of 2.5%

† *One unit of maltase activity releases 1 mgm. of glucose from maltose in 60 min. at a temperature of 30°C. (in the presence of a large excess of substrate).*
    One unit of amylase activity hydrolyzes 1 mgm. of Lintner starch to an arbitrary end point (1) in 60 min. at 30°C.

Lintner starch solutions were hydrolyzed with 1 ml. of an enzyme preparation having known maltase and amylase activities. The concentrations of glucose, maltose, and reducing dextrins* were determined at desired intervals. The results are summarized in Fig. 4. The effect of maltase and amylase in the enzyme preparations on the composition of the hydrolyzate can easily be observed with the use of the present analytical method. In the presence of a large excess of maltase, no maltose accumulates (Fig. 4,*A*). With higher enzyme activities, maltose accumulation begins only after the rate of glucose formation falls (Fig. 4,*B*). A slow rate of glucose formation with appreciable accumulations of reducing dextrins and maltose is obtained with an enzyme preparation having high amylase and a trace of maltase activity (Fig. 4,*C*).

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* *Reducing dextrins are expressed in milligrams of glucose having a reducing power equivalent to that of the dextrins determined with alkaline copper reagent.*

DETERMINATION OF GLYCEROL AND RELATED COMPOUNDS IN FERMENTATION SOLUTIONS BY PARTITION CHROMATOGRAPHY[1]

By A. C. NEISH

Abstract

A modified technique is described for applying partition chromatography, using a silica column, to the separation of simple organic compounds which are difficult to extract from water. An aqueous solution is pipetted directly on a Celite–water column the upper 15% of which has been left dry to receive it. Acetoin, 2, 3-butanediol, 1, 2-propanediol, ethanediol, glycerol, and erythritol can be separated from each other and from sugars and sugar alcohols containing five or more carbon atoms if the column is properly developed by ethyl acetate and benzene–butanol mixtures. After separation the compounds can be estimated by a rapid colorimetric determination of the acetaldehyde or formaldehyde formed by periodate oxidation. The recoveries are quantitative, thus making it possible to determine 1 to 2 mgm. of glycerol or 2, 3-butanediol within an error of ± 3% even when they are mixed with larger amounts of closely related compounds which cause interference in the usual methods.

Introduction

This work was undertaken primarily to obtain a specific method for determining glycerol in small amounts of fermentation solutions. Glycerol may be easily determined by oxidation with periodate followed by a colorimetric measurement of the formaldehyde formed (3). However, this procedure is not very specific, and compounds, such as mannitol, which frequently occur in fermentation solutions, may be determined and reported as glycerol.

The method described in this paper will not only differentiate between glycerol and mannitol but will even permit determination of glycerol when it is mixed with such closely related compounds as ethanediol and erythritol. The glycerol is separated from interfering substances by a new modification of the well known technique of partition chromatography on silica columns and then determined by the sensitive colorimetric method mentioned above. Other components in the mixture (i.e., 2, 3-butanediol) may be determined as well as glycerol, and the technique should prove useful for analysis of mixtures of simple water-soluble compounds such as are often found in fermentation solutions.

Experimental

Chromatography

Compounds like glycerol are difficult to transfer from water to solvents immiscible with water; consequently it was necessary to develop a technique whereby an aqueous sample could be pipetted directly into the column. The

method of packing the column described below makes it possible to analyze aqueous solutions directly and can be used to advantage even with compounds, such as the volatile fatty acids, that are easily extracted by ether.

Celite 535 was found to be a suitable form of silica for packing the column. It is sufficiently porous to enable viscous solvents such as n-butanol to be used as developing agents. The commercial product is purified by digestion with concentrated hydrochloric acid at room temperature overnight. It is then washed free of acid and dried in an oven. The Celite is purified for partition chromatography since it was found that certain compounds (i.e., acetic acid) could not be recovered quantitatively when commercial Celite was used.

The size of column and the packing technique are the same as those previously described (4). The Celite (3.0 gm.) is wetted with distilled water (3.0 ml.), slurried with the developing solvent, and compressed to a length of 7 to 8 cm. in the chromatogram tube. Another portion of dry Celite (0.5 gm.) is also slurried in the developing solvent and packed on top of this. Excess solvent is decanted and 0.5 ml. of water, containing the substances to be fractionated, is pipetted directly on the celite, care being taken to prevent any from getting on the walls of ،e tube except for 3 to 4 mm. above the surface of the Celite. The sample is washed in with two portions (1 ml.) of the developing solvent. This gives a Celite–water column in equilibrium with the developing solvent and containing the substances to be fractionated in the upper 15% of its length. It is developed using an appropriate solvent which is forced through the column, by air pressure, at the rate of three to four drops per second.

Analysis of Eluates

After chromatographic separation, the compounds (acetoin, 2, 3-butanediol, and glycerol) are obtained in organic solvents. It is necessary to transfer them back into water before they can be determined by the analytical procedures used. This is usually accomplished by addition of water and evaporation on a steam bath until only the aqueous phase remains. However, acetoin is rather volatile and it is preferable to partition it between the solvent and water and analyze the aqueous layer.

Acetoin may be eluted in 20 ml. of ethyl acetate. This fraction is shaken with an equal volume of water until equilibrium is reached. An aliquot of the aqueous phase is then suitably diluted and the acetoin determined colorimetrically by the creatine – alkaline alpha napthol reaction (6). Standard solutions of acetoin are partitioned in the same way and the aqueous phase used to obtain a calibration curve.

2, 3-Butanediol is usually obtained in 31 ml. of ethyl acetate. This is mixed with 20 ml. of water and evaporated on a steam bath with occasional stirring. The evaporation is discontinued as soon as the solution is monophasic. A suitable aliquot is then used for the colorimetric determination of the acetaldehyde formed on periodate oxidation, using the piperazine – nitroprusside reaction (1).

Glycerol can be eluted by 50 ml. of a benzene – *n*-butanol mixture containing 75% of *n*-butanol by volume. This is mixed with an equal volume of water and evaporated on a steam bath until about 15 to 20 ml. of the aqueous phase remains. The glycerol is then determined in a suitable aliquot by colorimetric determination of the formaldehyde formed on periodate oxidation (3). Other compounds, such as 1, 2-propanediol, ethanediol, and erythritol, which give formaldehyde on periodate oxidation, were also estimated by this procedure; a four hour oxidation period is used when monosaccharides are to be determined.

Results and Discussion

Since there is no rapid method of indicating the presence of compounds such as glycerol and the diols in the eluate, it is necessary to collect a number of successive fractions and then analyze them. The colorimetric methods of analysis used were chosen because they are more specific than some more general method such as the measurement of periodate consumed in oxidation.

The procedures were tested on known solutions in the presence of the developing solvents in order to see whether good results could be obtained. One rather unexpected result was the high loss of glycerol that occurs if insufficient water is present during evaporation of the solvent (see Table I). It is necessary to add a volume of water equal to the volume of solvent before placing the beaker on the steam bath.

TABLE I

GLYCEROL LOSSES ON EVAPORATION OF A SOLVENT

5.55 mgm. samples of glycerol in 1.5 ml. of water and 40 ml. portions of benzene + 75% *n*-butanol were added to a series of 250 ml. beakers. Varying amounts of water were then added and the samples evaporated to "dryness" on a steam bath

No.	Ml. of water added	Mgm. glycerol recovered	% glycerol recovered
1	nil	0.97	17.7
2	10	2.68	48.8
3	20	3.25	58.6
4	40	5.65	101.8
5	60	5.70	102.8

A great many trial experiments were run before a standard development procedure for the determination of glycerol and 2, 3-butanediol was decided on. It was found that 2, 3-butanediol could be recovered in good yields in a convenient volume of solvent when ethyl acetate was used as the developing agent. This solvent can also be used for recovery of 1, 2-propanediol which is removed more slowly than the 2, 3-butanediol and can be separated from it. Ethyl acetate is also a good solvent for the separation of acetoin and 2, 3-butanediol. For these reasons it was chosen as the first developing solvent. It is not a strong enough solvent to remove glycerol in a convenient volume, hence it was necessary to test other solvents for this purpose.

A mixture of benzene and *n*-butanol containing 75% of butanol by volume was found to remove glycerol at a convenient rate. It was also found to be suitable for the separation of glycerol from closely related compounds like ethanediol and erythritol (Table II).

TABLE II
SEPARATION OF GLYCEROL FROM SOME RELATED COMPOUNDS BY PARTITION CHROMATOGRAPHY

3.22 mgm. of 2, 3-butanediol, 1.85 mgm. of ethanediol, 1.92 mgm. of glycerol and 2.50 mgm. of *meso*-erythritol added to column

Fraction No.	Developing solvent	Ml. of solvent	Mgm. found	% Recovery
1	Ethyl acetate	20	Nil	
2	" "	31	3.16	98% of 2, 3-butanediol
3	Benzene–butanol (75%)	40	1.94	105% of ethanediol
4	" "	40	1.92	101% of glycerol
5	" "	5	Nil	
6	" "	5	Nil	
7	" "	10	0.75 ⎫	
8	" "	50	1.85 ⎬	104% of erythritol
9	" "	20	Nil ⎭	

These solvents were then used in a series of experiments on synthetic mixtures of acetoin, 2, 3-butanediol, and glycerol. The ethyl acetate was saturated with water just before use; the benzene–butanol mixture was also saturated by shaking with one-quarter volume of water and discarding the bottom phase. The development procedure, based on the preliminary work with single compounds, was standardized so that four successive fractions were collected as follows: (1) 20 ml. of ethyl acetate. (2) 31 ml. of ethyl acetate. (3) 40 ml. of benzene–butanol, and (4) 50 ml. of benzene–butanol. The acetoin is all in the first fraction, the 2, 3-butanediol all in the second, any ethanediol or 1, 2-propanediol present is all in the third, while the glycerol is all in the fourth fraction. Compounds such as mannitol remain in the column. It was found that this procedure gave reproducible results and good recoveries of acetoin, 2, 3-butanediol, and glycerol (Table III), even when substances such as etha-

TABLE III
ANALYSIS OF MIXTURES OF ACETOIN, GLYCEROL, AND 2, 3-BUTANEDIOL

No.	Mgm. added to column				Mgm. recovered			% recovered		
	Interfering substances*	Acetoin	2, 3-Butanediol	Glycerol	Acetoin	2, 3-Butanediol	Glycerol	Acetoin	2, 3-Butanediol	Glycerol
1	Nil	Nil	4.67	Nil	.....	4.65	....	.....	99.5	....
2	Nil	Nil	4.67	5.04	.....	4.68	5.00	.....	100.0	99.2
3	Nil	0.335	2.46	2.51	0.334	2.44	2.46	99.7	99.3	98.0
4	Mixt. I	Nil	2.46	2.51	.....	2.50	2.49	.....	101.5	99.4
5	Mixt. II	Nil	2.46	Nil	.....	2.45	....	.....	99.6	....
6	Mixt. III	Nil	2.46	2.51	.....	2.43	2.49	.....	99.0	99.4
7	Mixt. II	Nil	2.46	2.51	.....	2.44	2.56	.....	99.2	102.0
8	Mixt. III	Nil	2.46	2.51	.....	2.40	2.44	.....	97.6	97.3
9	Mixt. I	0.335	2.46	2.51	0.330	2.45	2.43	98.6	99.6	97.4
10	Mixt. II	0.335	2.46	2.51	0.338	2.45	2.50	101.0	99.6	99.6
11	Mixt. III	0.335	2.46	2.51	0.335	2.44	2.48	100.0	99.3	99.0

* *Mixture I   : 2.5 mgm. each of glucose, xylose, and mannitol added to the column.*
Mixture II : 2.5 mgm. of serine, threonine, glucose, xylose, and mannitol added to column.
Mixture III : 2.5 mgm. each of mannitol and sorbitol, 2.55 mgm. of ethanediol and 2.46 mgm. of 1, 2-propanediol added to the column.

nediol, 1, 2-propanediol, serine, threonine, glucose, xylose, and mannitol were present. Since these substances would interfere either in the determination of 2, 3-butanediol or glycerol by the colorimetric methods used, it is obvious that the chromatographic separations have greatly increased the specificity of these determinations.

This same development procedure was used in the analysis of several *B. subtilis* (Ford's type) and yeast fermentation solutions which are known to be free of appreciable quantities of interfering substances, except glucose. These solutions were analyzed by the colorimetric method directly and the glycerol found corrected for the glucose present. They were then analyzed using the chromatographic method, no corrections being made. The same results were obtained by both procedures (Table IV), within the usual limits of error.

TABLE IV

DETERMINATION OF GLYCEROL AND 2, 3-BUTANEDIOL
IN FERMENTATION SOLUTIONS

B. subtilis fermentations were run in a medium containing 5% glucose and 0.5% yeast extract; the yeast fermentations were carried out using a medium containing 22% glucose and 0.5% yeast extract

Solution fermented by	% residual glucose	% glycerol		% 2, 3-butanediol	
		Determined directly	Determined after chromatography	Determined directly	Determined after chromatography
B. subtilis.....	0.72	0.88	0.89	1.10	1.08
B. subtilis.....	0.36	0.99	0.98	1.32	1.28
Yeast.........	0.66	1.19	1.20	....	....
Yeast.........	0.64	1.20	1.18	....	....
Yeast.........	12.9	2.08	2.10	....	....

A chromatographic technique, such as the one described above has several advantages over conventional methods of analysis. Not only is it possible to analyze rather complex mixtures with simple reagents but closely related compounds can be separated from each other on a micro scale. Thus the technique can be applied to the isolation of small amounts of fermentation products in a relatively pure state. This should be useful in experiments with radioisotopes. For example, acetate labeled with isotopic carbon might be added to a bacterial fermentation and the various products separated by partition chromatography in order to see which ones have acquired carbon from the acetate.

It is possible for one worker to determine 2, 3-butanediol and glycerol accurately in four or five samples per day. This is rather slow for the examination of large number of cultures. However, it is more rapid than any other procedure of equal specificity and should be quite useful for checking the presence of glycerol in cultures where important amounts are found by a less specific, but more rapid method (3), which will not distinguish between glycerol and mannitol.

The technique described above could be applied to many mixtures other than the ones tried. Some unpublished experiments have given an almost complete separation of glucose and maltose when butanol–ethanol mixtures are used for developing the column. However, these compounds can be readily separated and determined quantitatively using paper chromatography (5). It has also been shown recently (2) that glycerol may be separated from the polyhydric alcohols in this way. The chief advantages of using a column are that volatile compounds may be recovered quantitatively and larger quantities can be handled. It would probably be impossible to separate acetoin, 2, 3-butanediol, and glycerol and recover each quantitatively in separate solutions by any other chromatographic procedure known to the author, although glycerol may have a low enough volatility to be handled quantitatively by paper chromatography provided it is not partially lost when the solvent is evaporated, as in the experiments reported in Table I.

Acknowledgment

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ON THE CAPACITY OF POROUS ALUMINUM OXIDE LAYERS[1]

By A. J. DEKKER AND HELEN M. A. URQUHART

Abstract

Porous aluminum oxide layers may be obtained by anodic oxidation in sulphuric acid. The base of the pores is separated from the metal by a thin insulating barrier layer. The experiments show that the ultimate thickness of the barrier layer remains constant after a critical value has been reached. The dependence of the final thickness on current density, concentration, and temperature has been investigated. It is suggested that an electronic current is involved in the mechanism which limits the growth of the barrier layer.

Introduction

It is well known that aluminum may be covered with an oxide layer by anodic oxidation in an appropriate electrolytic solution. From a macroscopic point of view, we can distinguish between porous and nonporous aluminum oxide layers. The former are obtained in electrolytes in which the oxide dissolves, for example, sulphuric or oxalic acid. Nonporous layers are formed in electrolytes that do not attack the oxide, for example, an ammonium borate solution. We will mainly be concerned with porous layers.

Photomicrographs suggest that these layers contain pores that are almost perpendicular to the aluminum plate (6). Values of the diameter of the pores as given in the literature vary between 0.01μ and 0.1μ (4, 5, 9). An indirect proof of the existence of the pores was provided by results obtained by Dekker and van Geel (5). These authors also found that the porosity of the oxide layer decreases for larger current densities. The photomicrograph in the paper by Edwards and Keller (6) shows clearly that the pores do not go right down to the metal but end in a thin barrier layer which covers the plate uniformly. Also, when an aluminum plate is oxidized with constant current in a sulphuric acid solution the voltage increases for a short time and then remains practically constant; this indicates the formation of a thin insulating layer during the period in which the voltage increases. This barrier layer separating the pores from the metal probably plays an important role in the oxide formation process (2). An idealized model of a porous layer is given in Fig. 1. The experi-
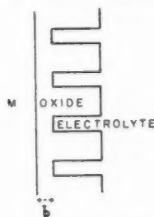

FIG. 1. *Idealized model of a porous aluminum oxide layer; b gives the thickness of the basic layer.*

ments described below are mainly concerned with the capacity of the layers. It was found that, when an aluminum plate was oxidized with a constant current in a sulphuric acid solution, the capacity reaches a "final" value in a short time and then remains constant during further passage of current. The dependence of the final capacity on current density, concentration of the solution, and temperature was investigated.

Experimental

Essentially the experiments are simple. An aluminum plate was oxidized with constant current in a solution of sulphuric acid, while the temperature was kept constant. Then, the capacity of the layer was measured. Each of these points will now be discussed in some detail.

The aluminum samples* used were square plates of 5 cm. sides, with a narrow strip extending above the electrolytic bath so that leads could be clipped on to the plates. The plates were cleaned with a 10% (by weight) solution of potassium hydroxide at room temperature and washed for a few minutes in distilled water. The plates were then oxidized in sulphuric or oxalic acid. The current was kept constant within about 1% by regulating the output of a d-c. supply by means of a potentiometer. The temperature was kept constant within 0.5°C. by using a cooling coil and a stirrer in the electrolytic bath. After the oxidation process the plates were washed again in distilled water and their capacity measured in a saturated solution of boric acid, to which ammonia had been added, giving a specific resistance of about 500 ohm-cm. (room temperature). The impedance measurements can not be carried out in sulphuric or oxalic acid because they dissolve the oxide.

The system aluminum oxide – electrolyte – counter electrode may in first approximation be represented by the equivalent circuit of Fig. 2. The oxide layer is represented by a capacity C and a nonlinear resistance R. The electrolyte gives rise to a series resistance r. The contact electrolyte – counter electrode is equivalent to a capacity C' and a parallel nonlinear resistance R'. From the measured impedance of this system the capacity of the oxide may be found. This procedure may be simplified in the following way: If C' is very large and R' very small, it is clear that the impedance of the whole cell practically becomes equal to the impedance of the CRr-system. In other words, the
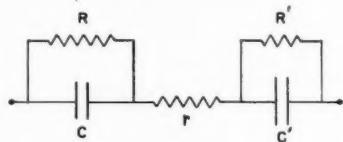
FIG. 2. *Equivalent circuit of the system Al – Al₂O₃ – electrolyte – counter electrode. For the meaning of the symbols see text.*

* *The aluminum was kindly provided by the Aluminum Co. of Canada Ltd. The samples used were Alcan 2S-0, being fully annealed Al with a purity between 99.0% and 99.49%. The estimated impurities as given by the Company are: 0.20% Cu, 0.10% Mn, 0.10% Zn, Fe and Si max. 1%.*

contact between the electrolyte and counter electrode becomes "perfect" in that case. This simplification was obtained by using a counter electrode with large area consisting of 10 sheets of tin foil folded into accordion plates and connected in series around the circumference of the bath.   The capacity of the tin foil–electrolyte interface thus obtained was 2400 μf. As all capacities measured were smaller than 66 μf. the error made in C by assuming a perfect contact between the tin foil and the ammonium borate solution was less than 1%, not even counting the low value of R'. This was considered as sufficiently accurate.

The impedance of the cell was measured by using a Philips capacity and resistance bridge (GM 4140 "Philoscope"). The accuracy is 1%. The circuit is given in Fig. 3. The cell of which the impedance has to be measured is repre-
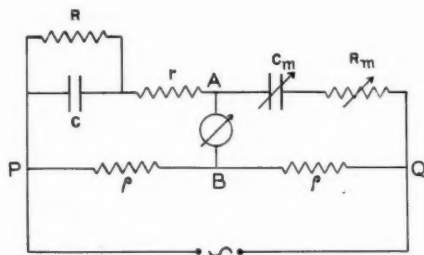
FIG. 3.   *Circuit for measuring the impedance of the oxidized aluminum plates.*

sented by a capacity C and a parallel resistance R, both in series with the resistance r of the ammonium borate solution. The contact tin foil–solution is assumed to be perfect for reasons mentioned above. The effective voltage across PQ is 2 v. As the polarization was very slight, 60 cycles could be used. Balance of the bridge is obtained by adjustment of the variable capacity C_m and the variable resistance R_m. Because there are two equal resistances ρ in branch PBQ, the impedance of the cell is given directly by the readings C_m and R_m. From these readings the capacity C of the oxide layer has to be calculated. This can easily be done in the following way. The impedance of the capacity C and the parallel resistance R alone, is equivalent to the impedance of the capacity C_m and a resistance $(R_m - r)$ in series. Hence

$$C = \frac{C_m}{1 + C^2_m (R_m - r)^2 \omega^2} \cdot$$

Now it was found by replacing the oxidized plate by a duplicate of tin foil that the series resistance of the ammonium borate solution was about 24 ohms. The value of $(R_m - r)$ could thus be estimated in each case. It turned out, however, that in none of the measurements the value of $C^2_m (R_m - r)^2 \omega^2$ exceeded 0.01, because $(R_m - r)$ was only a few ohms in all cases. Therefore the reading C_m on the variable capacitance was used as a sufficient approximation for the actual capacity of the oxide layer.

It may be remarked that the error made by assuming perfect contact between the tin foil and the electrolyte partially cancels the error made by using $C = C_m$.

Results

The most essential feature of the results, viz., that the capacity of the porous layers reaches a final value, is borne out by the following simple experiment. Seven different Al-plates, each with an area of 50 cm.^{2}, were oxidized in a 5.4% sulphuric acid solution* with a constant current of 200 ma. during different periods. The temperature was 14.5°C. The zero-capacity of each plate, that is, the capacity before oxidation, was larger than the maximum of the variable condenser (> 130μf.) in all cases. The final capacities are given in Table I. This table shows that, except for random fluctuations, the capacity is essentially the same in all cases and is independent of the time of oxidation after a certain time has elapsed. For the examples given, the critical time is apparently less than one minute.

TABLE I

THE FINAL CAPACITY OF SOME SAMPLES OXIDIZED FOR DIFFERENT PERIODS

Sample	Time of oxidation, min.	Final capacity, μf.
a	1	27.2
b	1	26.2
c	1	26.7
d	3	26.5
e	5	26.5
f	9	26.5

We found, however, that the final capacity of the oxide layers depends on the current density and on the concentration of the solution. Values obtained for the final capacity for different current densities and concentrations of sulphuric acid are plotted in Fig. 4. All these measurements were carried out at 14.5°C. Final capacities obtained in a saturated solution of oxalic acid are also given. It should be noted that for the lowest sulphuric acid concentration (1.2%) corrosion occurred at a current density of 8 ma. per cm^2. There was still a measurable capacity of about the value to be expected from a smooth curve, indicating that the layer is not short circuited. It is remarkable that the corrosion did not occur at higher temperatures. The authors cannot offer an explanation for this fact.

In Fig. 5 the results are plotted as the reciprocal final capacity against the logarithm of the specific resistance of the sulphuric acid solutions. The reciprocal final capacity actually is a measure of the final thickness of the basic layer (see discussion). From Fig. 5 we see that for concentrations lower than 9% the relation is linear and may be written as

* *% Refer to volume per cents through this paper.*

$$\left\{ \frac{\partial (1/C)}{\partial \log \rho} \right\} I, T \; = \; \text{constant (up to concentrations of about 9\%).}$$

The results show that this constant is practically independent of the current density for the range investigated.
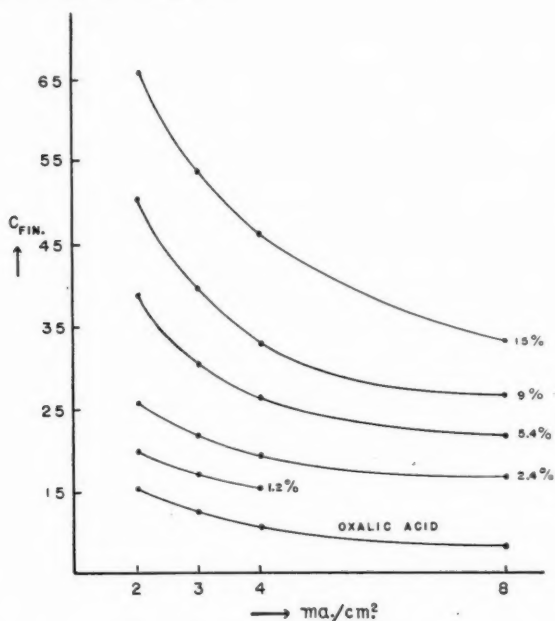
FIG. 4.   *The final capacity as a function of current density and concentration.*
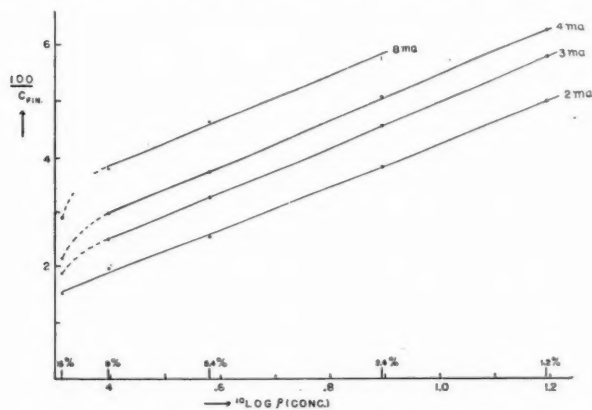
FIG. 5.   *The reciprocal of the final capacity as a function of the logarithm of the specific resistance of the sulphuric acid solution.*

A plot of $1/C$ against the logarithm of the current density also leads to a linear relation as may be seen from Fig. 6.   The slope of the curves obtained may depend slightly on the concentration.
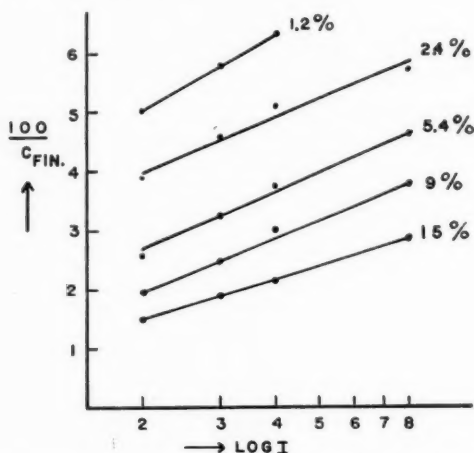
FIG. 6.   *The reciprocal of the final capacity as a function of the logarithm of the current density.*

The influence of temperature on the value of the final capacity was investigated between 13° and 44°C., using a 5.4% sulphuric acid solution and a constant current density of 4 ma. per cm². The results are given in Fig. 7 as a plot of the reciprocal final capacity against the logarithm of the specific resistance of the solution, which, in this case, is determined by the temperature. Although here again a straight line is obtained, the slope is much steeper than in Fig. 5. This means that the influence of temperature cannot be accounted for by a change in resistance alone. This may be partly due to an increase in solubility of the oxide.
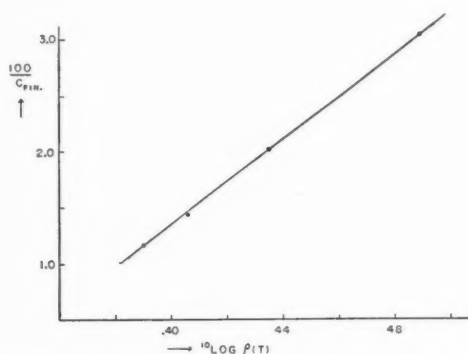
FIG. 7.   *The reciprocal of the final capacity as a function of the logarithm of the specific resistance as determined by temperature.*

The results may be summarized in the following way:

(1) $\left\{\dfrac{\partial 1/C}{\partial \log \rho}\right\}_{I,\,T} \simeq 0.04$ C in μf., ρ in ohm-cm. (for concentration up to 9%).

(2) $\left\{\dfrac{\partial (1/C)}{\partial \log I}\right\}_{T,\,\text{conc.}} \simeq 0.03$ C in μf., I in ma. per cm.^{2}

(3) $\left\{\dfrac{\partial (1/C)}{\partial \log \rho}\right\}_{I,\,\text{conc.}} \simeq 0.2$ C in μf., ρ in ohm-cm. (for a concentration of 5.4% and 4 ma. per cm.^{2}

Discussion

The first question that arises is, of course, the interpretation of the measured capacities. The capacity of a condenser depends on the geometry of the electrodes and on the dielectric constant of the material, in this case the oxide. From the structure of the layers indicated in Fig. 1 it is clear that the aluminum electrode may, in first approximation, be represented by a flat plate. The shape of the contact between the oxide and the electrolytic solution, however, is very complicated. The calculation of the capacity of such a system, even in the idealized form of Fig. 1, constitutes a highly involved mathematical problem. It is not likely that a solution of this problem would give a proportionate contribution to the understanding of the phenomena described in this paper. The essential point of the results is that the capacity reaches a final value after a relatively short time of oxidation. This indicates that the depth of the pores does not contribute appreciably to the capacity, although this will probably be the case for very small values of the depth. The main contribution to the capacity will come from the barrier layer; this seems reasonable in view of Fig. 1. For very large values of b (Fig. 1) one would expect the capacity to be equal to $C = \epsilon A / 4\pi b$, where A is the total area of the aluminum plate. For very small values of b one would expect $C = \epsilon A' / 4\pi b$, where A' stands for the total area of the pore bases. Experiments carried out by Dekker and van Geel (5) indicate, however, that the first formula is a good approximation.

In spite of the difficulties just mentioned, the following conclusion seems to be justified:

The final capacity corresponds to a final average thickness of the basic layer. The average thickness of the basic layer is approximately given by $b = \epsilon A / 4\pi C$, but may be smaller. Therefore $1/C$ as used in some of the graphs is approximately proportional to the thickness of the basic layer.

In connection with this last point, we may take a numerical example: For $\epsilon = 8$ (3), $A = 50$ cm.^{2} and $C = 34\mu$f., the corresponding final thickness becomes $b \simeq 10^{-6}$ cm.

The most intriguing problem is that of finding an explanation for the fact that the barrier layer does not increase in thickness after the "final" value has been reached. Closely related to this problem is, of course, the formation of the pores. Neither of these problems can be explained by the theory put forward by Verwey (12) and Mott (10). These authors assume that the oxide formation

is due to a migration of Al^{3+} ions from the metal to the oxide–electrolyte boundary under influence of the electric field. According to this idea, the oxide would grow at the oxide–electrolyte interface. However, only in the barrier layer is the electric field high enough to produce the observed ionic currents, and the formation of pores cannot be explained in this way. Anderson (2) pointed out that there is sufficient reason to assume a migration of both Al^{3+} and O^{2-} ions, leading to oxide formation at the metal–oxide interface. His theory does not exclude a growth also at the oxide–electrolyte interface, but whether or not this is the case will depend on the type of electrolyte used. According to Anderson, during the formation of the barrier layer at constant voltage, the field strength will decrease as the thickness increases, until a stationary state is reached in which the rate of growth is equal to the rate of acid attack. Once initiated at a number of points, the acid attack creates a situation similar to that of charged points opposite a plane conductor. This would cause the oxide to form in spherical caps around these points, thereby leading to a porous structure.

. In our experiments, however, the oxidation was carried out *not with constant voltage, but with constant current*. Hence, on the assumption that the observed current is completely ionic in character, it is difficult to see why, after the barrier layer has grown to a certain thickness, an equilibrium would be established between the rate of growth and the rate of acid attack.

A similar difficulty actually exists in the theory of the formation of nonporous layers: it is well known that when an aluminum plate is covered with a nonporous layer by anodizing it at constant current in an ammonium borate solution, the thickness of the layer increases with time and so does the voltage across the layer. When a critical voltage (thickness) has been reached, the growth of the layer stops, notwithstanding the fact that an electric current is flowing. Güntherschulze and Betz (8) found that the critical voltage depends on the concentration of the borate solution* according to

$$V_{crit} = - a \log c + \text{constant}.$$

As the specific resistance of the solution is in first approximation inversely proportional to the concentration c and as V is proportional to the thickness b of the layer, this relation may be written as

$$b_{crit} = a \log \rho + \text{const.} \tag{4}$$

It is evident that (4) is of the same form as our relation (1). Because chemical attack is excluded in the experiments of Güntherschulze, one has to assume that the current is carried by *electrons* as soon as the layer has reached its critical thickness. This is confirmed by the fact that sparks occur at this stage. Also, the luminescence observed during the oxidation process (1) indicates that there is always a small electronic current flowing during the oxidation process. The only possible explanation of the experiments just referred to seems to be that besides the ionic current, which leads to the oxide formation,

* Similar experiments have been carried out by van Geel (7).

an electronic current flows which is negligible for small values of the thickness, but increases strongly once the critical thickness is approached.

It is very remarkable that relation (4), obtained for the limit of growth of nonporous layers, is of the same form as our relations (3) and (1). It does not seem impossible therefore that an electronic current, which apparently limits the growth of nonporous layers, is also involved in the mechanism by which a limit is set to the thickness of the barrier of porous layers. It is clear, that if an electronic current does compete with the ionic current (which leads to oxide formation), an equilibrium may be set up as suggested by Anderson between the rate of growth of the barrier and the rate of acid attack. Once this stationary state was established, Anderson's reasoning would lead to the porous structure. Since the electronic current is determined by the number of electrons ejected from the negative ions in solution into the conduction band of the oxide, this current would increase with increasing conductivity of the electrolyte. Therefore, larger conductivity of the solution would lead to smaller values of the final thickness, in agreement with the experiments. Also, one would expect smaller values of the final thickness of the barrier layer for porous coatings than for nonporous ones (for the same conductivity of the solutions used), because in the formation of porous layers the acid attack has to be taken into account. An example may illustrate that this is confirmed by the experiments: Extrapolation of the curve for 3 ma. per cm.^{2} in Fig. 5 to a specific resistance of 400 ohm-cm. would give a corresponding final thickness of the barrier layer of 4×10^{-6} cm. An ammonium borate solution of the same specific resistance gives a nonporous coating with a final thickness of about 4.10^{-5} cm., which is about 10 times as large.

Supposing that the suggestion made above is correct, one still requires an explanation for the increase of the electronic current with increasing thickness of the barrier layer. This problem is related to the theory of rectification of electrolytic rectifiers. As no very satisfactory treatment of the latter problem is known at present, it seems a little premature to attempt a quantitative explanation of the effects observed in the present work. We may note, however, that according to the theory of dry rectifying contacts (11) an appreciable increase of the electronic current may be expected as soon as the thickness of the barrier becomes comparable with the mean free path of the electrons in the barrier, which is about 10^{-5}–10^{-6} cm. Since there are no free electrons in the solution, one cannot apply this theory directly to our problem. The mean free path, which is of the same order as the final thicknesses involved, may enter in the theory in another way: the electrons entering the oxide are accelerated by a field of about 2.10^7 v. per cm., which is much higher than the dielectric breakdown field of most ionic crystals. Once the thickness is large enough for collisions to occur, secondary electrons may be produced, thus leading to an avalanche. Actual breakdown would not occur because the number of primary electrons entering the oxide (determined by the concentration of negative ions in the solution and their electron levels) is relatively small.

Experiments are being carried out in this laboratory at present to investigate the above suggestions in more detail.

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THE DECOMPOSITION OF CUMENE HYDROPEROXIDE BY METAL SALTS AND COMPLEXES IN STYRENE SOLUTION[1]

By J. W. L. Fordham and H. Leverne Williams

Abstract

The rate of decomposition of cumene hydroperoxide in styrene at 30°C. is increased considerably by the presence of various metal salts and complexes. The rate increases in the presence of certain metal salts and complexes whether the metal is oxidizable, reducible, or incapable of undergoing a valence change. Both the metal and the nonmetal portion appear to influence the rate. The molar ratio of cumene hydroperoxide decomposed to metal complex added may be well over 1000. Most of the decomposition of cumene hydroperoxide in the presence of ferric phthalocyanine does not produce free radicals capable of initiating the polymerization of styrene and may proceed by an ionic chain mechanism.

Introduction

In current low temperature emulsion polymerization recipes, initiating free radicals are produced by the reaction between cumene hydroperoxide (CHP) and a metal complex. Typical recipes containing this type of initiating system have been reported recently (8, 10, 11). Wall and Swoboda (12) postulated that this reaction occurred in the oil phase between the peroxide and a ferrous soap. At present there is very little information in the literature on the effect of metal salts and complexes on the rate of decomposition of CHP. Kolthoff and Medalia (6) and the authors (4) have found that ferrous iron speeds up its rate of decomposition considerably in aqueous solutions. However, there is more information available on the reactions between metal salts and complexes and other peroxides. Chromous, cuprous, ferrous, ferric, manganous, mercury, and titanous ions (1, 7) and ferrous, ferric, cobalt, and chromium phthalocyanine (2) accelerate the rate of decomposition of hydrogen peroxide. Ferric iron has a somewhat weaker effect than ferrous iron (7) and the effect of the phthalocyanines decreases in the following order: ferrous > ferric > cobalt and chromium. Complex compounds of copper with ammonia, pyridine, piperidine, and glycine are very active in increasing the rate of decomposition of hydrogen peroxide, and copper compounds are more effective than the corresponding complex compounds of cobalt, nickel, and silver (9). Tetralin peroxide is decomposed rapidly by manganese, cobalt, and lead oleates (13). The present study is a preliminary examination of the effect of metal salts and complexes on the rate of decomposition of CHP in styrene.

Source of Chemicals

Styrene and CHP—as described previously (3).

2, 2'–Bipyridyl and o-phenanthroline monohydrate—reagent grade; G. F. Smith Co.

Ferric phthalocyanine—laboratory sample MDD 514; E. I. du Pont de Nemours.

Copper phthalocyanine—Monastral Blue: Canadian Industries Limited.

Linoleates, naphthenates, oleate, and resinates—technical grade; Harshaw Chem. Co.

Complexes containing bipyridyl or phenanthroline, ferrous pyrophosphate, ferric pyrophosphate, ferric stearate, and ferric laurate—synthesized from laboratory pure chemicals.

Remaining metal salts, hemin, and dihydroxyacetone—laboratory pure chemicals.

Technique

The same general procedure was followed as outlined in the previous study of the effect of shortstops on the initiator (3) except that a solution containing 1.0×10^{-4} mole (or as indicated) of metal salt or complex per kilogram of styrene was used in place of the shortstop. When 2, 2'–bipyridyl, o-phenanthroline monohydrate, or dihydroxyacetone was used, the total amount (combined and free) added was 5.0×10^{-4} mole per kgm. The initial CHP concentration was 0.2 mole per kgm. unless otherwise stated. The reactants were sealed in evacuated tubes. Hydroperoxide concentration was determined by the iodometric method used previously (3). Conversion of styrene monomers to polystyrene was measured by coagulating the polystyrene in a hot 90–10 ethanol–water mixture and weighing the dried residue.

Results

The effect of various styrene-soluble metal salts and complexes on the rate of decomposition of CHP at 30.25°C. is given in Table I.

There was no decomposition after 300 min. in the presence of the insoluble compounds ferrous pyrophosphate, ferrous bipyridyl chloride, ferric laurate, ferric pyrophosphate, hemin, silver bipyridyl complex, zinc bipyridyl chloride, and dihydroxyacetone. Also no decomposition occurred in the presence of the soluble complexing agents bipyridyl and phenanthroline alone.

The work indicated that exceedingly small quantities of ferric phthalocyanine accelerate the decomposition of CHP at low temperatures. If polystyrene of molecular weight 100,000 were formed and one mole of peroxide sufficed to initiate and terminate each chain then the decomposition of about 5% of the hydroperoxide should polymerize the solvent completely. Although this was not observed in the experiment in which ferric phthalocyanine was used as complex, in contrast with the results in the study of the thermal decomposition (3), it was thought that this compound might function as an activator in low temperature emulsion polymerization recipes (8, 10, 11) thereby reducing the amount of iron in the recipe and presumably in the copolymer. The copolymer might then show improved aging properties. However at 4.45°C. only

TABLE I

EFFECT OF METAL SALTS AND COMPLEXES ON THE RATE OF CHP DECOMPOSITION

Metal salt or complex	Solubility	CHP reacted, % initial weight	
		60 min.	300 min.
Ferric phthalocyanine................	s.	34.1	66.7
Cuprous chloride + P...............	s.	42.9	63.1
Cupric bipyridyl chloride..........	s.	38.4	52.8
Cuprous chloride + B...............	s.	39.6	46.0
Cobalt linoleate....................	s.	12.6	23.9
Zinc chloride + P..................	p.s.	0.0	23.9
Ferrous chloride + P..............	p.s.	4.0	11.0
Copper naphthenate................	s.	2.5	10.9
Cupric chloride + P...............	s.	4.5	9.4
Copper resinate....................	s.	2.0	9.2
Copper phthalocyanine.............	p.s.	0.7	6.2
Copper oleate.....................	s.	0.7	6.2
Copper naphthenate + B..........	s.	2.6	3.8
Ferric chloride + P...............	p.s.	1.2	3.5
Ferric stearate + P...............	p.s.	−0.3	2.2
Silver nitrate + P................	s.	0.2	1.5
Ferric stearate....................	p.s.	0.0	1.5
Iron naphthenate + P.............	s.	0.0	1.5
Zinc naphthenate..................	s.	−1.8	1.5
Iron linoleate.....................	s.	1.0	1.0
Iron naphthenate..................	s.	0.0	1.0
Lead linoleate.....................	s.	0.2	0.8
Iron resinate......................	s.	0.0	0.2

NOTE:—*P   —o-phenanthroline monohydrate.*
B   —2, 2'-bipyridyl.
s   —soluble to the extent of at least 1×10^{-4} mole per kgm. styrene.
p.s.—partially soluble, less than 1×10^{-4} mole dissolved per kgm. styrene.

very low bulk conversion of styrene to polystyrene occurred in the presence of ferric phthalocyanine after as much as 20 hr. although the CHP did undergo decomposition (Table II).

The polymerization that was observed occurred at low rates of CHP decomposition; this might be taken to indicate that the rate of initiation was too great for conversion of monomer to high polymer except at longer reaction times. However, the results in Table III indicate that this is only a partial reason for this lack of conversion, because the change in the conversion is not

TABLE II

STYRENE POLYMERIZATION AT 4.45°C.

Reaction time, hr.	CHP reacted		% Conversion
	% Initial weight	% Per hour	
1.00	19.7	19.7 0–1 hr.	0.0
2.00	36.4	16.7 1–2 hr.	0.0
4.00	53.6	8.6 2–4 hr.	0.7
20.00	71.1	1.1 4–20 hr.	8.1

TABLE III

EFFECT OF INITIATOR CONCENTRATION ON 20 HR. CONVERSION AT 4.45°C.

CHP, mole/kgm. sol'n.	Ferric phthalocyanine, mole/kgm. sol'n.	% Conversion
6.55×10^{-3}	0.07×10^{-4}	3.3
	0.33	4.2
	0.66	3.3
	6.55	0.0
6.50×10^{-2}	0.07	5.7
	0.33	7.5
	0.65	7.5
	6.50	0.0

appreciable when the rate of initiation, as governed by ferric phthalocyanine concentration and CHP concentration, is varied over a rather wide range extending down to a relatively slow rate.

The lower CHP concentration is similar to that used in commercial emulsion recipes (8) for the production of cold rubber. Use of such a polymerization recipe with ferric phthalocyanine or copper naphthenate as sole activator did not yield any copolymer, in agreement with the above data for the bulk phase polymerization.

Discussion

The results in Table I indicate that the metal does not have to be in its reduced state in the salt or complex in order to increase the rate of CHP decomposition in styrene. Indeed it would seem from the rate of decomposition in the presence of the zinc complex of phenanthroline that the metal does not have to undergo a valence change. The decomposition in the presence of oxidizable or reducible metal salts may be a special type of a more general case which includes all metals whether they are able to change valence or not. Kern (5) has also presented evidence to support this view. Furthermore both the metal and the nonmetal portion appear to influence the rate of hydroperoxide decomposition because if either is changed the rate is changed. The addition of complexing agent to metal salt decreases the rate in the case of bipyridyl and copper naphthenate.

It will be noted from Tables I and II that the rate of decomposition of CHP by rapid catalysts diminishes rapidly with time, and there is evidence that the reaction stops before complete destruction of the CHP is reached. The rate with slower catalysts shows an induction period which may be still longer with the "ineffective" materials so that it exceeds the total experimental time.

Of interest is the fact that 0.142 mole of CHP is decomposed by 0.0001 mole of ferric phthalocyanine with the polymerization of but 0.77 mole of styrene. This is an apparent kinetic chain length for styrene polymerization of only 4 to 5 monomer units. Also the decomposition of CHP must be a chain reaction of considerable persistence; over 1400 moles are decomposed per mole of ferric

phthalocyanine. Since there was no gas pressure in the tubes comparable to the amount of peroxide decomposed, the mechanism of decomposition must also include this fact.

From the results in Tables II and III it is probable that most of the decomposition of CHP in the presence of ferric phthalocyanine takes place by a mechanism that does not yield free radicals capable of initiating polymerization. It would appear that the use of ferric phthalocyanine as an activator is not feasible in free radical initiated polymerizations.

Acknowledgments

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THE APPLICATION OF THE ARNDT–EISTERT REACTION TO THE SYNTHESIS OF FATTY ACIDS OF HIGH MOLECULAR WEIGHT[1]

By F. A. Vandenheuvel and P. Yates[2]

Abstract

The Arndt–Eistert reaction offers a convenient method for the synthesis of the higher members of the aliphatic carboxylic acid series. Nonadecanoic acid, eicosanoic acid, and heneicosanoic acid have been prepared successively from stearic acid in good yields. An efficient method of purification of the synthetic products is described. The ultraviolet absorption maxima for some diazoketones derived from the higher members of the aliphatic carboxylic acid series are recorded.

Little is recorded in the literature on the application of the Arndt–Eistert reaction to aliphatic carboxylic acids. The sole reference in the fatty acid series is to the conversion of acetic acid to propionic acid (4, 12); in addition, two members of the saturated dicarboxylic acid series have been subjected to bishomologation by this method (10, 13). The present work describes the preparation of nonadecanoic, eicosanic, and heneicosanic acids from octadecanoic acid. Each of the three necessary steps (5) involved in this type of synthesis: acid → (I) acid chloride → (II) diazoketone → (III) next higher homologue acid, have been studied in order to find out the methods most expeditious and leading to the highest yields. The best method of purification of the crude product has also been worked out. The following paragraphs summarize these findings.

I. Preparation of Acid Chlorides

The method described by Adams and Ulich (1) and the method described by Bauer (6) were both found adequate. The acid chloride is thus prepared by the action on the acid of either an excess of thionyl chloride purified by the method of Cottle (7) or an excess of oxalyl chloride; this is followed by the removal of the excess reagent under vacuum, on the water bath. Attempts to purify further the acid chloride by vacuum distillation have proved not only unnecessary but always wasteful.

II. Preparation of the Diazoketone

The method used is similar to one already described by Grundmann (9). The diazomethane is prepared by the method of Arndt (2). An example of how to work out the reaction mixture is given in the experimental section of this paper (1 diazo-2-nonadecanone). It was found that the crude product could be used as such for the next step. The yield in diazoketone, calculated on the acid used, was better than 90%.

III. *Conversion of the Diazoketone*

The direct rearrangement of the diazoketone into the next higher acid in 1, 4-dioxane, using silver oxide as catalyst (10), was unsuccessful. Repeated attempts led only to products different from the carboxylic acid sought. The use of ammonia and silver oxide was also found unsatisfactory.

The conversion of the diazoketone into the next higher acid ester was successful and led to good yields. As far as yields were concerned, two methods were found equally satisfactory. The first effects the conversion in absolute ethanol using silver oxide as catalyst (3). The second effects the conversion in benzyl alcohol at higher temperature (11); in this case no catalyst is necessary. The latter method is certainly more expeditious. However, a detailed description of the first method is also given in the experimental section (nonadecanoic acid). Both methods lead finally to an 80% yield in crude acid.

IV. *Purification of the Synthetic Acids*

It was found that a small amount of a colored impurity could never be removed from the crude acids either by recrystallization from various solvents or by treatment with Norit. Purification was very efficient, however, when the following steps were observed:

   (i) One recrystallization from 90% ethanol,

  (ii) Evaporation and condensation at a pressure of 0.5 micron,

 (iii) One recrystallization from 90% ethanol.

The acids obtained by this procedure melted over a small range of temperature and their melting points were in some cases very close to the highest recorded (8). Their elementary analyses and neutralization values (milligrams of potassium hydroxide for 1 gm. acid) were satisfactory.

The melting points and ultraviolet absorption of the intermediate diazoketones have been recorded. In all cases a characteristic maximum at a wave length very close to 248 mμ was obtained.

In order to shorten the present paper, specific examples have been selected from the complete data and are presented in the experimental section below. Their purpose is to describe in detail the methods outlined above.

Experimental

All melting points are corrected and were determined by using a Herschberg apparatus, equipped with a calibrated Anschütz thermometer; the rise in temperature did not exceed 0.3° per min.

Octadecanoic (Stearic) Acid

"Pure" stearic acid (Eastman Kodak 402) was recrystallized four times from 80% aqueous ethanol including two treatments with Norit. The product had a melting point of 69.6°C. (recorded by Francis and Piper (8): m.p. of stearic acid 69.6°C.).

Stearyl Chloride

Pure stearic acid (60 gm., m.p. 69.6°) and 60 ml. of thionyl chloride, purified by the method of Cottle (7), were refluxed gently for two hours, then vigorously for one hour. Excess reagent was removed under reduced pressure. The product was used without further purification.

1-Diazo-2-nonadecanone

An ethereal solution of stearyl chloride (1 mole) was added slowly with shaking to a cooled ethereal solution of diazomethane (2.5 moles) prepared from N-nitrosomethylurea by the method of Arndt (2). After standing for 12 hr., ether was removed on the water bath under reduced pressure until solid began to separate; the mixture was then cooled at −20°C. The deposit was filtered off and washed with a little cold ether. The product was freed of ether by standing in air and finally dried *in vacuo* over phosphorus pentoxide. The crude diazoketone was obtained in quantitative yield as a yellow solid and was used as such for the next preparation.

A sample recrystallized twice from dry ether gave lemon yellow leaflets, m.p. 69.1°C. (reported by Grundmann (9) m.p. 69°C.).

The ultraviolet absorption spectrum of a 0.002% solution in isopropanol showed maximum at $\lambda = 247$ mμ; $\epsilon = 10,700$.

Nonadecanoic Acid

To a solution of 1-diazo-2-nonadecanone (31 gm.) in absolute ethanol (310 ml.) at 55–60°C., was added silver oxide in small portions (8 gm. total) over a period of 25 hr. with mechanical stirring; after the first seven hours, the temperature was raised to 60–65°C. and after a further 12 hr. to 65–70°C. When addition of silver oxide was complete, a sample of the reaction mixture no longer evolved nitrogen when treated with hydrochloric acid; the mixture was then boiled gently under reflux for three hours. It was filtered hot and the filtrate left to cool; the small amount of yellow solid separating was removed by filtration. The filtrate was hydrolyzed by boiling with ethanolic potassium hydroxide (17 gm. potassium hydroxide). Acidification and extraction with ether gave the crude acid as a cream colored powder, m.p. 65–66°C., in 83.5% yield. Purified by the method described below, it yielded white glistening leaflets, m.p. 68.5°C. (beginning of fusion 67.8°C.) (recorded by Francis and Piper (8) for nonadecanoic acid: m.p. 68.65°C. Calc. for $C_{19}H_{38}O_2$: C, 76.50; H, 12.84%. Found: C, 76.58; H, 12.63%. Neutralization equiv. (milligrams potassium hydroxide per gram acid): calc., 187.9; found, 187.2.

1-Diazo-2-eicosanone

This was prepared from nonadecanyl chloride by the method described above for 1-diazo-2-nonadecanone.

The chloride had not been distilled and gave the diazoketone in 88% yield calculated on the nonadecanoic acid used. A sample, twice recrystallized from

dry ether, gave lemon leaflets, m.p. 71.2°C.: ultraviolet absorption spectrum of 0.002% isopropanol solution showed maximum at $\lambda = 247$ mμ and $\epsilon = 10,200$

The diazoketone was used without purification for the next step.

Eicosanic acid

1-Diazo-2-eicosanone (9 gm.) was added in small portions, with mechanical stirring to 100 ml. benzyl alcohol heated in an oil bath at 175°C. When addition was complete and nitrogen evolution had ceased, the mixture was gently boiled under reflux for a further 15 min. After removal of the excess benzyl alcohol, the residual product was recrystallized from 95% aqueous ethanol; yield of benzyl eicosanate, 83% (a sample twice recrystallized from 95% ethanol gave colorless needles; m.p. 50.2°C.).

The benzyl ester was hydrolyzed with ethanolic potassium hydroxide and after acidification, ether extraction, and evaporation of the extract the free acid was obtained as a pale yellow powder in over-all yield of 80% (based on the nonadecanoic acid used).

When purified (see procedure below) the crude acid gave white glistening leaflets, m.p. 75.2°C.; beginning of fusion 74.2°C. (recorded by Francis and Piper (8) for eicosanic acid: m.p. 75.35°C.). Calc. for $C_{20}H_{40}O_2$: C, 76.85; H, 12.90%. Found: C, 77.02; H, 12.78%. Neutralization value (milligrams potassium hydroxide for 1 gm. acid): calc., 179.5; found, 179.9.

Eicosanyl Chloride

Eicosanoic acid (5 gm.; m.p. 74.2–75.2°C.) and 6 ml. oxalyl chloride were heated under reflux at 70–80°C. for four hours. Excess oxalyl chloride was removed under reduced pressure on the steam bath and the chloride was left as a pale yellow liquid: this was used without purification.

1-Diazo-2-heneicosanone

The crude diazoketone was obtained as a yellow solid by removal of ether from the reaction mixture of eicosanyl chloride and diazomethane in ether. The yield, calculated from the acid chloride was quantitative. A sample three times recrystallized from dry ether gave very pale yellow leaflets, m.p. 73.5°C.; absorption maximum in isopropanol solution at 249 mμ. Calc. for $C_{21}H_{40}ON_2$: C, 74.94; H, 11.98%. Found: C, 74.93; H, 12.16%.

Heneicosanic acid

1-Diazo-2-heneicosanone rearranged in hot benzyl alcohol gave an 80% yield of the crude heneicosanoic acid.

The purified acid (see below) was obtained in white glistening leaflets, m.p. 72.0–73.5°C. (recorded by Francis and Piper (8) for heneicosanic acid: m.p. 74.3°C.). Calc. for $C_{21}H_{42}O_2$: C, 77.25; H, 12.96%. Found: C, 77.36; H, 12.76%. Neutralization value (milligrams potassium hydroxide for 1 gm. acid): calc., 171.7; found, 170.2.

Purification of Synthetic Acids

Before the distillation in high vacuum of the crude acids, it was found necessary to submit them to one recrystallization from 90% ethanol, in order to remove impurities which decompose at the temperature of the distillation and may considerably hamper the process.

The distillations were effected in an apparatus very similar in design to an ordinary vacuum sublimation apparatus but with a wide (25 mm. diam.) side tube leading to the pump to minimize the pressure drop; a good oil diffusion pump produced the vacuum. The pressure inside the apparatus was 0.5 micron. The bath was maintained at temperatures varying with the acid involved: 90°C. for nonadecanoic acid, 110°C. for eicosanic acid, 130°C. for heneicosanic acid.

The distance between the molten acid and the condensing surface was 15 mm. The apparatus could handle 3 gm. of substance at a time; when the residue was reduced to a few drops of liquid, fresh acid was added and the operation resumed. By so doing, small losses were encountered and apparently most of the impurities were retained in the residue which was deeply colored; the distillate, however, was a pure white solid. One final recrystallization from 90% alcohol was effected mainly to homogenize the different distillates, since it was found that a slight difference in the melting point could be detected between the first portions condensed and the last. The whole process of purification could be effected with only 10% loss.

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SYNTHESIS OF PARA-BRIDGED BENZENE COMPOUNDS[1]

By K. Wiesner, D. M. MacDonald, R. B. Ingraham, and R. B. Kelly

Abstract

2-5 Tetradecamethylene and 2-5 decamethylene phthalic anhydride have been obtained by dehydrogenation of the corresponding tetrahydro compounds. A 10-membered para-bridged acyloin, VI, has also been obtained in 70% yield by ring closure. From these results it is concluded that it should be possible to synthesize compounds with para-bridges containing less than 10 carbons.

In a previous communication (1) we described the synthesis of maleic anhydride addition products of large ring dienes, which we have prepared with the intention of converting them into 2–5 polymethylenephthalic acids by dehydrogenation. These dehydrogenation experiments have now been completed and are reported in the present communication.

In order to get experience with mild dehydrogenations of this kind we have prepared 2, 5-diethyltetrahydrophthalic anhydride, I, by addition of 1, 4-diethylbutadiene (2) and maleic anhydride. The addition product was easily purified by distillation and served as a model in the dehydrogenation studies. A very suitable procedure for our purpose was found in the dehydrogenation process devised by Linstead *et al.* (3).

In this method the compounds are dehydrogenated by palladium on charcoal in the medium of boiling naphthalene in a current of carbon dioxide. The progress of the reaction can be followed by measuring the volume of hydrogen produced after absorbing the carbon dioxide in potassium hydroxide solution.

In this way we have obtained 2, 5-diethyl, 2-5 decamethylene and 2-5 tetra-decamethylene phthalic anhydrides (II, IIIb, and IVb) as beautiful crystalline compounds in very good yield.

As proof of structure we have oxidized 2-5 tetradecamethylenephthalic anhydride with potassium permanganate and obtained 1, 2, 3, 4-benzene-tetracarboxylic acid. Its identity was proved by melting point and mixed melting point of the beautifully crystalline tetramethyl ester with an authentic specimen (5).

The absorption spectra of II, IIIb, and IVb were taken in 0.01 N alcoholic sodium hydroxide solution. They show a maximum at 280 μ. The same maximum is also obtained with phthalic acid. As the length of the para-bridge exerts no detectable influence on the position of the maximum, it seems that the 10-membered para-bridge is still unstrained.

We have been able to corroborate this conclusion by performing an acyloin ring closure (4) on the diester, V, prepared according to the directions of

Formula scheme

I   II

IIIa (n = 10)
IVa (n = 14)

IIIb (n = 10)
IVb (n = 14)

$$CH_3OOC - (CH_2)_4 - \overset{}{\bigcirc} - (CH_2)_4 - COOCH_3$$

V

VI   VII

VIII   IX
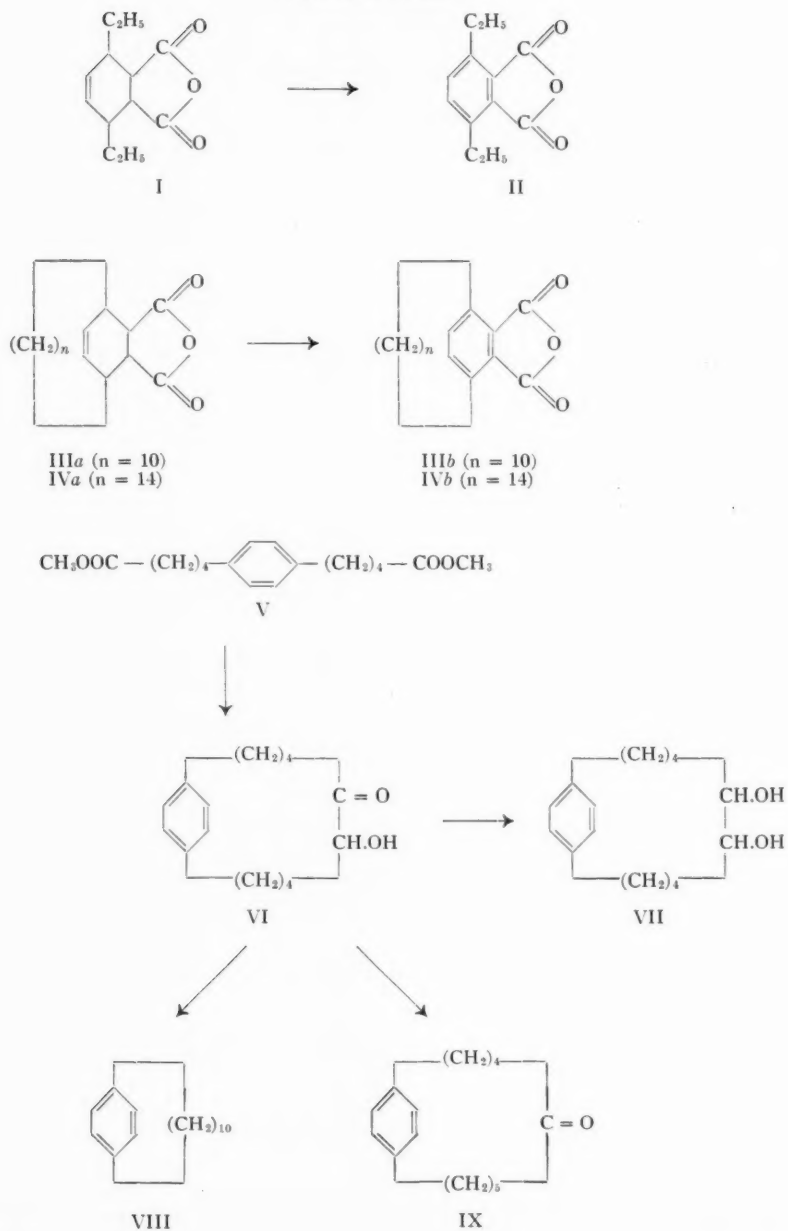
Ruzicka and collaborators (6). The corresponding acyloin, VI, has been obtained in 70% yield. The acyloin can be smoothly converted into the diol, VII, either by hydrogenation with platinum oxide in alcohol or by reduction with lithium aluminum hydride. By treatment of the acyloin with acetic and hydrochloric acids and zinc a mixture of 1–4 decamethylenebenzene, VIII, and the ketone, IX, was obtained. The ketone was separated in the form of the semicarbazone and the hydrocarbon was purified by fractional distillation over sodium.

The high yield of the acyloin ring closure seems to indicate that not only is there no strain in the 1-4 decamethylene bridge on the benzene nucleus but that the presence of the benzene ring increases the cyclization tendency. It is worth mentioning that this yield is 12% larger than the yield of the 14 carbon ring acyloin under identical conditions.

As the result of these investigations it may be concluded that the smallest possible para-bridge will probably have less than 10 carbon atoms. Attempts to synthesize the 9 and 8 membered bridges are in progress.

Experimental

2, 5-Diethyltetrahydrophthalic Anhydride

Diethylbutadiene (2), (4.78 gm.) and 10 gm. of maleic anhydride were refluxed in 300 cc. of dry toluene under pure nitrogen for eight hours. The toluene was removed by distillation. The residue was treated with 20 gm. of potassium hydroxide in 200 ml. of water, warmed to 50°C. and allowed to stand overnight. The alkaline solution was extracted with ether, acidified, and the precipitated acid extracted with ether. The ether solution was washed several times with water, dried, and the solvent distilled off. The acid obtained in this way was converted into the anhydride by distillation. The anhydride boiled at 150°C. (8 mm.), (yield: 7.56 gm.). It melted at 45°C. and was redistilled for analysis. Calc. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.75%.   Found: C, 69.17; H, 7.86%.

2, 5-Diethylphthalic Anhydride

The dehydrogenation was performed in an apparatus described by Linstead *et al.* (3). 2-5 Diethyltetrahydrophthalic anhydride (301 mgm.) was dehydrogenated with 260 mgm. of 30% palladium charcoal and 1 gm. of naphthalene at the boiling point of naphthalene. The theoretical volume of hydrogen was produced in six hours and then the gas evolution stopped. The contents of the reaction vessel was dissolved in ether and the catalyst filtered off. The ether was evaporated and the product stirred with 10 cc. of a 10% potassium hydroxide solution overnight. The alkaline solution was extracted with ether to remove the naphthalene, acidified, and the precipitated acid extracted with ether. After drying and distilling off the ether, the crude acid was converted into the crystalline anhydride by sublimation. (Yield: 248 mgm.). The melting point after repeated recrystallizations from hexane was 122–125°C. It was found very difficult to obtain accurate and reproducible analytical data on this

anhydride presumably owing to its easy conversion to the acid. It was there-
fore characterized as the anilide. This compound melted after five crystal-
lizations from ethanol at 60–61°C. and was sublimed for analysis in high
vacuum.   Calc. for $C_{18}H_{17}NO_2$: C, 77.39; H, 6.13; N, 5.02%. Found: C, 77.23;
H, 6.05; N, 5.09%.

2-5 Tetradecamethylenephthalic Anhydride (IV b)

The tetrahydro compound IVa (201 mgm.) was dehydrogenated with 240
mgm. of palladium charcoal and 1 gm. of naphthalene exactly as in the pre-
vious experiment. It gave the theoretical amount of hydrogen in six hours and
was worked up as described before. The yield of the crystalline anhydride after
sublimation was 141 mgm. It was recrystallized eight times from hexane and
melted sharply at 118°C. For analysis it was sublimed in high vacuum at 90°C.
It sublimed in large beautifully developed prisms.   Calc. for $C_{22}H_{30}O_3$: C,
77.15; H, 8.83%.   Found: C, 77.20; H, 8.98%.

2-5 Decamethylenephthalic Anhydride (III b)

In exactly the same way, 183 mgm. of the tetrahydro compound, IIIa, was
converted into 108 mgm. of the dehydrogenated anhydride, IIIb. After six
crystallizations from ether – petroleum ether it melted at 107°C. It was sub-
limed for analysis in high vacuum at 90°C.   Calc. for $C_{18}H_{22}O_3$: C, 75.49; H,
7.74%.   Found: C, 75.33; H, 7.88%.

Oxidation with Permanganate

2-5 Tetradecamethylenephthalic anhydride (0.922 gm.) was suspended in
30 ml. of water and a solution of 11.8 gm. of potassium permanganate in 300 ml.
of water was added with stirring in the course of two hours. The solution was
then heated to 80°C. and stirring continued for 24 hr. After this time the
solution was acidified and a current of sulphur dioxide passed through it until
it was decolorized. It was then continuously extracted with ether. The ether
extract was extracted with a 5% sodium hydroxide solution; this alkaline
extract was strongly acidified and extracted continuously with ether.

After distilling off the ether, 98 mgm. of a crystalline acid was obtained. It
was recrystallized three times from hydrochloric acid diluted with an equal
amount of water. The acid was esterified with diazomethane. The crystalline
ester was recrystallized from ether and after sublimation melted at 128–130°C.
A sample of 1, 2, 3, 4-benzenetetracarboxylic tetramethylester (5) melted at
129–131°C. A mixture of both melted at 128–130°C.

Acyloin Ring Closure

The diester, V, (33.1 gm.), was cyclized in an apparatus described by Prelog
et al. (4) using the high dilution technique. It was dissolved in 300 ml. of pure
dry xylene and added over a period of 13 hr. to an emulsion of 11 gm. of sodium
in 1500 ml. of dry xylene kept at 112°C. and stirred at very high speed. The

rate of addition was controlled by means of an adjustable leveling bulb containing mercury. The whole operation was done under dry and carefully purified nitrogen. After the addition was completed, the stirring was continued for two hours. At the end of this time 200 ml. of methanol was added, and this was followed by 20% sulphuric acid until the mixture was acid. The xylene layer was separated, washed with water, 5% carbonate solution and water, dried, and the solvent distilled off in vacuum. The oily residue distilled from a Hickman flask at 0.3 mm. 150–160°C. The yield was 18.62 gm., which is 70% of the theoretical. For analysis it was redistilled from a collar flask and the middle fraction analyzed. Calc. for $C_{16}H_{22}O_2$: C, 78.01; H, 9.00%. Found: C, 78.07; H, 8.93%. After standing in the icebox the whole product solidified to a very low melting crystalline solid.

Reduction to the Diol VII

On hydrogenation in alcohol with platinum oxide the acyloin, VI, took up one mole of hydrogen. After filtering off the catalyst and evaporation to dryness, the diol was obtained in quantitative yield as a colorless oil which slowly crystallized in clusters of beautiful long needles. It was recrystallized seven times from ethyl acetate – hexane and melted at 102–103°C. For analysis it was sublimed in high vacuum at 90°C. Calc. for $C_{16}H_{24}O_2$: C, 77.37; H, 9.74%. Found: C, 77.39; H, 9.73%.

Titration with 0.1 N periodic acid: 8.1 $\times$ 10^{-4} mole of diol consumed 7.6 $\times$ 10^{-4} mole of periodic acid in five minutes.

The same compound was also obtained by lithium aluminum hydride reduction of the acyloin.

Reduction with Zinc and Hydrochloric acid

The acyloin, VI (1.6 gm.), was heated with 4 ml. acetic acid, 4 ml. concentrated hydrochloric acid, and 4 gm. of zinc to 100°C. for two hours. In the course of this time three more portions of 4 ml. of hydrochloric acid were added at half hour intervals. Then the reaction mixture was cooled, diluted with water, and extracted with ether. The ether extract was washed with water, sodium carbonate and water, dried, and the solvent removed by distillation. From the crude residue a semicarbazone was prepared, and this was separated from the hydrocarbon by extraction with petroleum ether in which the hydrocarbon dissolved. The crude semicarbazone of the ketone, IX, was obtained in a yield 0.561 gm. (30% of the theoretical). The semicarbazone was crystallized by dissolving in benzene and precipitating with hexane. It melted at 141–144°C. It was recrystallized five times for analysis and dried in high vacuum at 90°C. Calc. for $C_{17}H_{25}ON_3$: C, 71.04; H, 8.77; N, 14.62%. Found: C, 71.06; H, 8.81; N, 14.55%.

The hydrocarbon, VIII, was purified by distillation over sodium. It boiled in a collar flask at 133–143°C. (outside temperature) at 3 mm. The yield was

0.845 gm. (68% of the theoretical). For analysis it was redistilled over sodium, and the middle fraction was analyzed.   Calc. for $C_{16}H_{24}$: C, 88.82; H, 11.18%. Found: C, 88.69; H, 10.71%.

Acknowledgment

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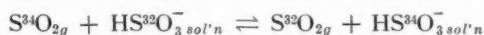# THERMODYNAMIC PROPERTIES OF ISOTOPIC COMPOUNDS OF SULPHUR[1]

By A. P. Tudge and H. G. Thode

Abstract

Using the well known methods of statistical mechanics, partition function ratios for many isotopic sulphur compounds have been calculated. These partition function ratios are used to determine equilibrium constants for many possible exchange reactions involving the isotopes of sulphur. The results indicate that considerable fractionation of the sulphur isotopes can be expected in laboratory and natural occurring processes. The predicted exchange constants are discussed in the light of recent results on the distribution of the sulphur isotopes in nature.

Recent investigations in this laboratory (14) have shown that relatively large variations in the abundances of the sulphur isotopes occur depending on the source of the sulphur. These variations amount to 5% for S^{34} and 10% for S^{36}. On the basis of the evidence, it is reasonable to assume that fractionation of the sulphur isotopes has occurred in natural biological and geological processes owing to differences in the chemical and physical properties of isotopic sulphur compounds.

H. C. Urey and his coworkers (18) used the exchange reactions

$$S^{34}O_{2g} + HS^{32}O_{3\,sol'n}^{-} \rightleftharpoons S^{32}O_{2g} + HS^{34}O_{3\,sol'n}^{-}$$

$$S^{36}O_{2g} + HS^{32}O_{3\,sol'n}^{-} \rightleftharpoons S^{32}O_{2g} + HS^{36}O_{3\,sol'n}^{-}$$

to separate the sulphur isotopes in a countercurrent extraction system. The exchange constants for these two reactions have since been determined directly (13) and are 1.019 and 1.039 respectively, favoring the heavier isotopes in the liquid phase. Aside from these reactions, however, little has been known about isotope exchange processes involving sulphur.

It is well known, of course, that the isotopes of other light elements do differ in their chemical properties and that isotope fractionation can occur in chemical reactions. Since the energy, entropy, and free energy of isotopic substances depend on the vibrational frequencies of the molecules, which in turn depend on the masses of the atoms in the molecules, it can be seen that there is a theoretical basis for the differences in the chemical properties of isotopic substances. That these effects should be appreciable and result in isotope fractionation in laboratory and natural occurring processes was first shown by H. C. Urey and L. Greiff (17). Their early calculations have since been confirmed by experiment. Recently H. C. Urey (15) made a comprehensive theoretical study of many possible chemical exchange processes involving the isotopes of H, B, C, N, O, Li, and the halogens. In each case, partition function

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ratios for many isotopic substances were calculated. These calculations have been extended to isotopic compounds of sulphur, and the results are discussed in the light of the extensive variations found in the abundances of the sulphur isotopes in terrestrial sources.

Theoretical

Equilibrium Constants for Exchange Reactions

H. C. Urey (15) has recently used a relatively simple method for the determination of partition function ratios of isotopic molecules. By a combination of these ratios, equilibrium constants for specific exchange reactions are obtained. This method is essentially as follows.

A typical exchange reaction may be written as:

$$aA_1 + bB_2 \rightleftharpoons aA_2 + bB_1, \qquad\qquad \text{E(1)}$$
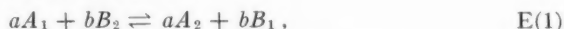
where A and B are molecules which have some one element as a common constituent and subscripts 1 and 2 indicate that the molecule contains only the light or the heavy molecule respectively. It is well known that the equilibrium constant K for any reaction is given by

$$-RT.\ln.K = \Delta F^\circ \qquad\qquad \text{E(2)}$$

where F° is the standard free energy. For a reaction of the type E(1), one obtains

$$-RT.\ln.K = aF^0_{A_2} + bF^0_{B_1} - aF^0_{A_1} - bF^0_{B_2}. \qquad\qquad \text{E(3)}$$

The free energy is related to the partition function by the following equation:

$$F = E_0 + RT.\ln.N - RT.\ln.Q, \qquad\qquad \text{E(4)}$$

where E_0 is the "zero point energy" of the molecule,

N is the Avogadro number, and

Q is the partition function of the molecule.

On substituting E(4) in E(3) and simplifying, one gets the relation

$$K = \left[\frac{Q_{A_2}}{Q_{A_1}}\right]^a \Big/ \left[\frac{Q_{B_2}}{Q_{B_1}}\right]^b \; e^{-\frac{aE_{0A_2} + bE_{0B_1} - aE_{0A_1} - bE_{0B_2}}{RT}} \qquad\qquad \text{E(5)}$$

Instead of taking E_0 as the "zero point energy" (which is unique for any molecule), one can take E_0 as the bottom of the "potential energy curve" for the molecule. Since the potential energy curves are practically identical for isotopic molecules, then E_{0A_2} equals E_{0A_1}, and E_{0B_2} equals E_{0B_1}. Thus the exponential term in E(5) becomes unity, and

$$K = \left[\frac{Q_{A_2}}{Q_{A_1}}\right]^a \Big/ \left[\frac{Q_{B_2}}{Q_{B_1}}\right]^b. \qquad\qquad \text{E(6)}$$

Therefore, in order to calculate the value of K, it is first necessary to determine the Q_2/Q_1 ratio for substances A and B.

The ratio Q_2/Q_1 for a chemical compound (diatomic) is given by the equation

$$\frac{Q_2}{Q_1} = \frac{I_2}{I_1} \frac{\sigma_1}{\sigma_2} \frac{M_2^{3/2}}{M_1^{3/2}} \frac{e^{-\frac{u_2}{2}} (1 - e^{-u_1})}{(1 - e^{-u_2})\ e^{-\frac{u_1}{2}}} ,$$

E(7)

where σ_2 and σ_1 are the symmetry numbers of the two molecules (identical for all compounds studied in this paper), M_2 and M_1 are their molecular weights, u_2 and u_1 are related to the fundamental vibrational frequencies by relations of the kind $u_i = hc\omega i/kT$, and I_2 and I_1 are the moments of inertia.

The following reasoning is then used by H. C. Urey (15) to obtain further simplifications. If the right and left sides of Equation E(7) are multiplied by $(m_1/m_2)^{3/2n}$ (where m_1 and m_2 are the atomic weights of the isotopic atoms being exchanged and n is the number of isotopic atoms being exchanged) and if the right side is multiplied and divided by the ratio $\dfrac{u_1}{u_2}$, it is possible to simplify this expression.

Thus E(7) becomes

$$\frac{Q_2}{Q_1} \left[\frac{m_1}{m_2}\right]^{\frac{3}{2n}} = \frac{\sigma_1 u_2\ e^{-\frac{u_2}{2}} (1 - e^{u_1})}{\sigma_2 u_1\ (1 - e^{u_2})\ e^{-\frac{u_1}{2}}} ,$$

E(8)

since

$$\frac{I_2 M_2^{3/2}\ m_1^{\frac{3}{2n}}\ u_1}{I_1 M_1^{3/2}\ m_2^{\frac{3}{2n}}\ u_2} = 1$$

E(9)

according to the Teller and Redlick theorem. New partition functions are then defined where

$$\frac{Q_2'^{*}}{Q_1'} = \frac{Q_2}{Q_1} \left[\frac{m_1}{m_2}\right]^{\frac{3}{2n}} .$$

E(10)

It is obvious that the equilibrium constant K is given by

$$\left[\frac{Q_{A_2}'}{Q_{A_1}'}\right]^a \left[\frac{Q_{B_2}'}{Q_{B_1}'}\right]^b = K .$$

E(11)

Equation E(8) can be put in a more convenient form for purposes of calculation. Defining

$$x = \frac{u_1 + u_2}{4} \text{ and } \delta = \frac{u_1 - u_2}{2}$$

E(12)

and expanding in terms of δ, E(8) becomes

$$\ln \frac{Q_2'}{Q_1'} = \ln \frac{\sigma_1}{\sigma_2} + \ln \frac{u_2}{u_1} + (\coth x)\delta.$$

E(13)

Our Q's and Q''s are just the reverse of those used by H. C. Urey.

Although the entire preceding treatment has been given for diatomic molecules, it is possible to go through similar reasoning for polyatomic molecules and obtain equations similar to E(12) and E(13).

$$\ln \frac{Q_2'}{Q_1'} = \ln \frac{\sigma_1}{\sigma_2} + \sum_i \ln \frac{u_{2i}}{u_{1i}} + \sum_i (\coth x_i)\, \delta_i \qquad\qquad \text{E(14)}$$

$$x_i = \frac{u_{2i} + u_{1i}}{4}; \; \delta_i = \frac{u_{1i} - u_{2i}}{2} \qquad\qquad \text{E(15)}$$

u_i are related to the various fundamental vibrational frequencies (in cm.$^{-1}$) of the isotopic molecules as shown above. Therefore, although the calculation for a single partition function would be extremely complicated, the *partition function ratio* for *isotopic molecules* is easily obtained from a *knowledge of vibrational frequencies alone.*

In order to determine Q_2'/Q_1', it is necessary to obtain the vibrational frequencies of the molecules containing the most abundant isotope from spectroscopic data and then to calculate the frequencies of the rare isotopic molecules by means of well known "normal vibration equations". This method must be used because, in general, the rare isotopic molecule is in such low concentration that its vibrational frequencies cannot be experimentally determined.

"Normal vibration equations" have been known for many molecules for some time and these equations approximately relate the frequencies to "force constants" and atomic weights (8). If one assumes these same "force constants" to hold for both the abundant and the rare molecule, then it is possible to calculate frequencies for both isotopic molecules (by putting in the appropriate atomic weights) and then find the differences in the frequencies. While these calculated frequencies may be slightly in error (since the normal vibration equations are only approximate), the *differences* can be evaluated quite accurately by this method. Using these differences and the experimentally observed fundamental frequencies for the abundant molecule, it is possible to determine the fundamental frequencies for the molecule containing the rare isotope.

As pointed out by Urey, the partition functions for polyatomic molecules may be slightly in error because only the fundamental frequencies are known (i.e., no anharmonic terms). Also, errors will be introduced when we apply statistical mechanical formulas relating to ideal gaseous substances to condensed phases or solids in solution.

Isotope Effects in Unidirectional Processes

Considerable work has been done recently on the experimental determination of the effect of isotopes on the specific reaction rates. These researches have mainly been on the decomposition of organic compounds. It has been shown conclusively (2, 9, 11, 19) that isotopic molecules have different rates of reaction and that, in general, the molecule containing the lighter isotope reacts faster.

The problem of reaction rates has also been approached from a theoretical point of view. Eyring (6) and Evans and Polanyi (5) have treated rate reactions from a statistical viewpoint and their method is called "theory of absolute rates". J. Bigeleisen (2) in a recent paper has considered the ratio of rate constants for competitive reactions of isotopic molecules and has derived the following formula which gives the approximate ratio of the rate constants.

$$\frac{k_1}{k_2} = \frac{Q_{R_2}}{Q_{R_1}} \bigg/ \frac{Q_2^{\dagger}}{Q_1^{\dagger}} \left[\frac{m_2^*}{m_1^*}\right]^{\frac{1}{2}} . \qquad E(16)$$

The k's are the specific rate constants, Q_R and $Q\dagger$ are the partition functions for the reactants and "activated complex" respectively, m^* is the effective mass of the activated complex, and the subscripts 1 and 2 refer to the light and heavy isotopic species respectively.

Thus we see that the k_1/k_2 ratio really depends on the differences in chemical properties of the isotopes (as determined by the partition function ratios). However, it is not yet possible to calculate k_1/k_2 because the values of the partition function ratios for the activated complexes are unknown. Also, it is not at all certain what values should be given to m_2^*/m_1^*.

Results

Table I contains a list of the frequencies of the diatomic molecules used in the calculations, while Table II gives the corresponding data on the polyatomic molecules.

The value of the partition function ratios and equilibrium constants are given in Table III. The method of tabulation in Table III is the same as that employed by Urey. The Q_2'/Q_1' ratios are given at two temperatures, while the equilibrium constants are contained at the intersection of the appropriate row and column. In all cases, the heavy isotope will concentrate in the compound listed at the left.

TABLE I

MOLECULAR FREQUENCIES

Molecule	ω_e	$\omega_e x_e$*	Reference
PbS^{32}	428.14	1.20	Herzberg. I. (7, p. 491)
PbS^{34}	417.10	1.14	Calculated. Herzberg. I, p. 71, 151
$S^{32} - S^{32}$	725.8	2.85	Herzberg. I, p. 492
$S^{34} - S^{34}$	704.2	2.70	Calculated. Herzberg. I, p. 71, 151
$S^{32} - S^{34}$	715.1	2.77	Calculated. (Note that the symmetry number is not equal to those for S^{32}–S^{32} and S^{34}–S^{34}.)

* *The vibrational frequency used to calculate a Q_2'/Q_1' ratio is ω where $\omega = \omega_e - 2\omega_e x_e$.*

TABLE II

MOLECULAR FREQUENCIES

(The numbers in brackets represent the degree of degeneracy of a frequency)

Mole-cules	Frequencies				References
	w_1	w_2	w_3	w_4	
H_2S^{32}	2611	1290	2684		Herzberg. II (8, p. 170)
H_2S^{34}	2610.77	1287.90	2679.45		Calculated. Herzberg. II, p. 169
H_2S^{36}	2610.57	1286.02	2675.38		" " " "
$S^{32}CN^-$	2065.53	398.18 (2)	752.81		Calculated. Herzberg. II, p. 174
$S^{34}CN$	2064.99	397.58 (2)	743.02		" " " "
$S^{32}CO$	2081.82	529.24 (2)	859.09		Calculated. Herzberg. II, p. 174
$S^{34}CO$	2080.47	528.42 (2)	847.77		" " " "
CS_2^{32}	655.79	396.55 (2)	1522.64		Calculated. Herzberg. II, p. 172
CS_2^{34}	636.24	394.70 (2)	1515.56		" " " "
$S^{32}O_2$	1151	519	1361		Herzberg. II, p. 285
$S^{34}O_2$	1142.84	514.93	1343.75		Calculated. Herzberg. II, p. 169
$S^{32}O_3$	1069	652	1330 (2)	532 (2)	Herzberg. II, p. 178
$S^{34}O_3$	1069	640.40	1310.65 (2)	530.04 (2)	Calculated. Herzberg. II, p. 178
$S^{32}O_4^=$	980	451 (2)	1113.60 (3)	618.90 (3)	Urey and Bradley (17), p. 1977
$S^{34}O_4^=$	980	451 (2)	1097.56 (3)	615.55 (3)	Calculated. Urey and Bradley
$S^{36}O_4^=$	980	451 (2)	1083.21 (3)	612.30 (3)	" " " "
S_8^{32*}					
S_8^{34}					

*S_8^{32}	470	216	267	557	465	(2)	200	(2)	243	(2)	434	(2)
S_8^{34}	455.98	209.56	259.04	540.39	451.13	(2)	194.04	(2)	235.75	(2)	421.06	(2)
S_8^{32}	152	(2)	542	(2)	185	(2)						
S_8^{34}	147.47 (2)	525.84 (2)	179.48 (2)	*Bhagavantam and Venkatarayudu (1)*								

Perhaps special reference ought to be made to the Q_2'/Q_1' ratio of 1.000 for $S^=$ ion. The rigorous partition function ratio for a molecule which has only translational degree of freedom is $Q_2/Q_1 = (M_2/M_1)^{3/2}$, where M_1 and M_2 are the molecular weights of the heavy and light molecules respectively. Following Urey's method of simplification (outlined earlier), one obtains

$$\frac{Q_2}{Q_1}\left[\frac{m_1}{m_2}\right]^{3/2n} = \left[\frac{M_2}{M_1}\right]^{3/2}\left[\frac{m_1}{m_2}\right]^{3/2n}. \qquad E(17)$$

In the case of $S^=$ ion, n equals unity, and M equals m so that when new partition functions are defined one obtains

$$\frac{Q_2'}{Q_1'} = \frac{Q_2}{Q_1}\left[\frac{m_1}{m_2}\right]^{3/2n} = 1.000 . \qquad E(18)$$

By analogy, the values of Q_2'/Q_1' for many monatomic ions and molecules should be unity. Values for Li^+, Cl^-, Br^-, and I^- could be added to Urey's tables of exchange constants.

It is interesting to note that the enrichment factors $(K - 1)$ for exchange involving S^{36} are twice those involving S^{34}. This is seen in the following reactions:

$$S^{32}O_4^- + H_2S^{34} \rightleftharpoons S^{34}O_4^- + H_2S^{32} \quad K \text{ (calc.)} = 1.074 \text{ at } 25°C.$$
$$S^{32}O_4^- + H_2S^{36} \rightleftharpoons S^{36}O_4^- + H_2S^{32} \quad K \text{ (calc.)} = 1.145 \text{ at } 25°C.$$
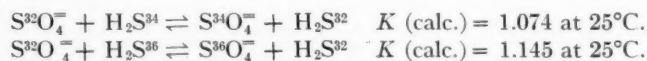
TABLE III

EQUILIBRIUM CONSTANTS FOR SULPHUR EXCHANGES

	$\frac{S^{34}O_4^=}{S^{32}O_4^=}$	$\frac{S^{34}O_3}{S^{32}O_3}$	$\frac{S^{34}O_2}{S^{32}O_2}$	$\frac{S^{34}CO}{S^{32}CO}$	$\left[\frac{CS_2^{34}}{CS_2^{32}}\right]^{\frac{1}{2}}$	$\left[\frac{S_8^{34}}{S_8^{32}}\right]^{\frac{1}{8}}$	$\frac{S^{34}CN^-}{S^{32}CN^-}$	$\left[\frac{S_2^{34}}{S_2^{32}}\right]^{\frac{1}{2}}$	$\frac{H_2S^{34}}{H_2S^{32}}$	$\frac{PbS^{34}}{PbS^{32}}$	$\frac{S^{34=}}{S^{32=}}$	°C.
$\frac{Q_2'}{Q_1'}$	1.101	1.096	1.053	1.022	1.021	1.018	1.016	1.015	1.015	1.010	1.000	0
	1.088	1.084	1.045	1.019	1.019	1.016	1.014	1.013	1.013	1.009	1.000	25
$\frac{S^{34}O_4^=}{S^{32}O_4^=}$	1.000	1.005	1.046	1.077	1.078	1.082	1.084	1.085	1.085	1.090	1.101	0
		1.004	1.041	1.068	1.068	1.071	1.073	1.074	1.074	1.078	1.088	25
$\frac{S^{34}O_3}{S^{32}O_3}$		1.000	1.041	1.072	1.073	1.077	1.079	1.080	1.080	1.085	1.096	0
			1.037	1.064	1.064	1.067	1.069	1.070	1.070	1.074	1.084	25
$\frac{S^{34}O_2}{S^{32}O_2}$			1.000	1.030	1.031	1.034	1.036	1.037	1.037	1.043	1.053	0
				1.026	1.026	1.029	1.031	1.032	1.032	1.036	1.045	25
$\frac{S^{34}CO}{S^{32}CO}$				1.000	1.001	1.003	1.006	1.007	1.007	1.012	1.022	0
					1.000	1.003	1.005	1.006	1.006	1.010	1.019	25
$\left[\frac{CS_2^{34}}{CS_2^{32}}\right]^{\frac{1}{2}}$					1.000	1.003	1.005	1.006	1.006	1.011	1.021	0
						1.003	1.005	1.006	1.006	1.010	1.019	25
$\left[\frac{S_8^{34}}{S_8^{32}}\right]^{\frac{1}{8}}$						1.000	1.002	1.003	1.003	1.008	1.018	0
							1.002	1.003	1.003	1.007	1.016	25
$\frac{S^{34}CN^-}{S^{32}CN^-}$							1.000	1.001	1.001	1.006	1.016	0
								1.001	1.001	1.005	1.014	25
$\left[\frac{S_2^{34}}{S_2^{32}}\right]^{\frac{1}{2}}$								1.000	1.000	1.005	1.015	0
									1.000	1.004	1.013	25
$\frac{H_2S^{34}}{H_2S^{32}}$									1.000	1.005	1.015	0
										1.004	1.013	25
$\frac{PbS^{34}}{PbS^{32}}$										1.000	1.010	0
											1.009	25
$\frac{S^{34=}}{S^{32=}}$											1.000	0
												25

Discussion and Conclusions

Distribution of S^{34} in Nature

It is quite obvious from the results in Table III that the isotopic compounds of sulphur do differ in their chemical properties and that fractionation of the isotopes will occur in equilibrium and unidirectional processes. The results obtained on the distribution of S^{34} in nature (14, 12, 10) are summarized in graphical form in Fig. 1. It is seen from the figure that sulphates as a group are enriched in S^{34} as compared to other sources of sulphur and that H_2S is in general depleted in S^{34}. Qualitatively this is what one would predict from the calculated partition function ratios, because the heavier isotope always concentrates in the compound with the higher Q_2'/Q_1' ratio (see Table III). In this connection it is interesting to note that in general those compounds
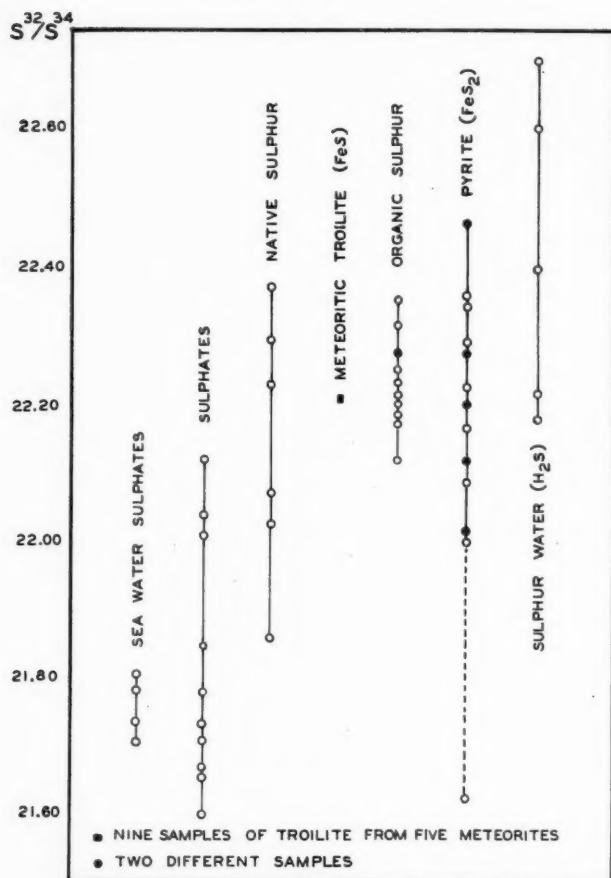
FIG. 1.*

where the sulphur is in a higher valence state have higher Q_2'/Q_1' ratios. This same result was obtained by H. C. Urey (15) in relation to partition function ratios for chlorine compounds.

	$\dfrac{Cl^{37}O_4^-}{Cl^{35}O_4^-}$	$\dfrac{Cl^{37}O_3^-}{Cl^{35}O_3^-}$	$\dfrac{Cl^{37}O_2}{Cl^{35}O_2}$	$\left[\dfrac{Cl_2^{37}}{Cl_2^{35}}\right]^{\frac{1}{2}}$	$\dfrac{HCl^{37}}{HCl^{35}}$	$\dfrac{Cl^{37}-}{Cl^{35}-}$
$\dfrac{Q_2'}{Q_1'}$	1.097	1.055	1.036	1.009	1.005	1.000

	$\dfrac{S^{34}O_4^=}{S^{32}O_4^=}$	$\dfrac{S^{34}O_3}{S^{32}O_3}$	$\dfrac{S^{34}O_2}{S^{32}O_2}$	$\left[\dfrac{S_2^{34}}{S_2^{32}}\right]^{\frac{1}{2}}$	$\dfrac{H_2S^{34}}{H_2S^{32}}$	$\dfrac{S^{34}=}{S^{32}=}$
$\dfrac{Q_2'}{Q_1'}$	1.101	1.096	1.053	1.015	1.015	1.000

* *Taken from paper by J. MacNamara and H. G. Thode. Phys. Rev. 78: 307. 1950.*

Samples of ground water which were rich in both sulphate and dissolved hydrogen sulphide were investigated previously. In each case the sulphate was enriched in S^{34} as compared to the hydrogen sulphide. This enrichment varied from 2.2 to 4.5%. It is seen, however, from the partition functions in Table III that the exchange constant for the equilibrium reaction

$$H_2S^{34}{}_g + S^{32}O_4^{=}{}_{sol'n} \rightleftharpoons H_2S^{32}{}_g + S^{34}O_4^{=}{}_{sol'n}$$
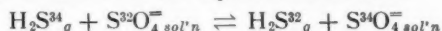
is 1.074 at 25°C.

Also, it has been found that native sulphur associated with hydrogen sulphide in sulphur water wells has the same isotopic content as the hydrogen sulphide. This is what one would predict from the exchange reaction

$$H_2S^{34} + \tfrac{1}{2}S_2^{32} \rightleftharpoons H_2S^{32} + \tfrac{1}{2}S_2^{34},$$

since the calculation equilibrium constant for this process is 1.000.

Sea water sulphates are of considerable interest. All samples analyzed to date are enriched in S^{34} and the concentration falls in the range with sulphate deposits (Fig. 1). Although there is a significant variation in the S^{34} content of water samples from the Atlantic, Pacific, and Arctic, the variation is nevertheless small and the S^{34} content of the oceans would seem to be fairly constant. Further, the fact that gypsum deposits in Ontario laid down from the sea about 350 million years ago have very nearly the same isotopic content as sea water sulphates of today indicates that the isotopic abundances of sulphur in sea water have been fairly constant over a long period of time. In this connection there are large native sulphur deposits around the Gulf of Mexico which presumably have been formed from ocean sulphates by bacterial action. These sulphur deposits contain 3% less S^{34} than sea water sulphates, which compares with a theoretical value of 8% if the bacterial processes were carried out under equilibrium conditions.

Although the isotope fractionation is in the direction and of the order of magnitude as that predicted by the theoretical calculations, nevertheless it is difficult to postulate a mechanism whereby isotopic equilibrium between $SO_4^{=}$ and H_2S may be established. To obtain an exchange between H_2S and $SO_4^{=}$, it would be necessary to have conditions that would both reduce the sulphates and oxidize the hydrogen sulphide. Although it is unlikely that sulphates could be reduced in nature by ordinary chemical agents, it is known that certain anaerobic bacteria reduce sulphates to hydrogen sulphide. If those bacteria reduce $SO_4^{=}$ in a unidirectional manner, then it would be necessary for other agents (aerobic bacteria) to oxidize H_2S in order for a cyclic interchange (exchange) to take place. On the other hand, if these bacteria reduce $SO_4^{=}$ to H_2S and at the same time oxidize H_2S back again to $SO_4^{=}$, then these agents alone would produce an exchange. Controlled laboratory experiments on the bacterial reduction of sulphates are now in progress in this laboratory in an attempt to determine the mechanism.

It is realized, of course, that although the exchange constants calculated from theoretical consideration predict in many cases the isotope fractionation found
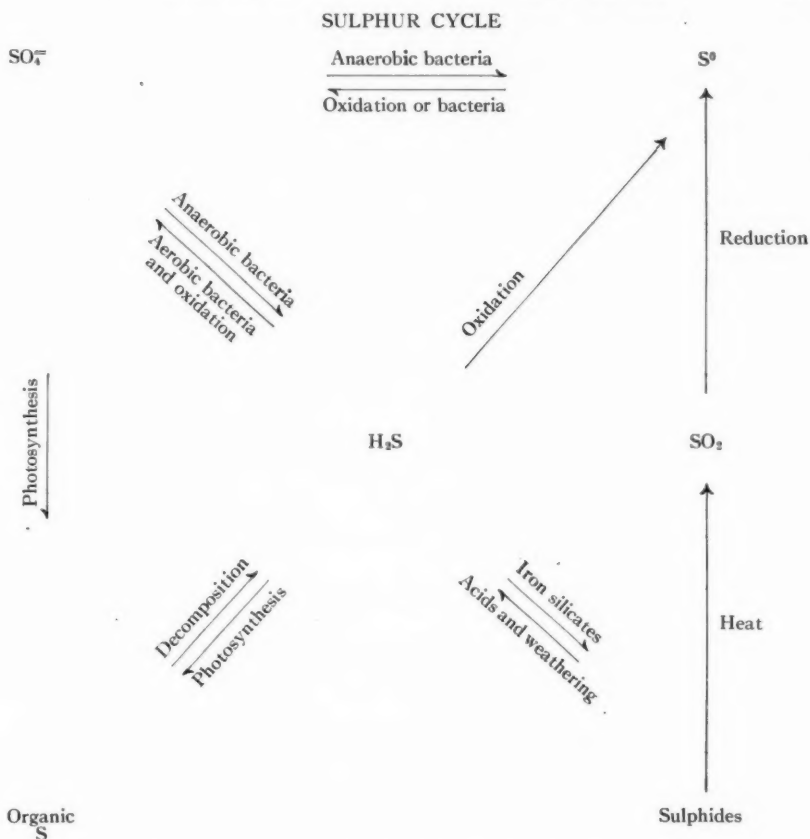
in nature, quantitative agreement cannot be expected. This is true since in nature many physical and chemical processes are involved and in some cases there is complete conversion of sulphur from one form to another without any over-all change in isotopic composition. However, the products of one reaction may become the reactants in another reaction, and so there is a chance that the slight fractionations may be additive, resulting finally in widespread variations in isotopic abundances.

It should be pointed out that fractionation of the isotope of the light elements may occur in unidirectional as well as equilibrium processes. Recently, Yankwich, and Calvin (19) have reported an isotope effect in the decarboxylation of malonic acid, the CO_2 being depleted in C^{13}. Also, Lindsay, McElcheran, and Thode (9) showed that C^{13} concentrates in the CO_2 in the dissociation products of oxalic acid. In other words, it is not necessary to have an equilibrium process for isotope fractionation to occur. Certainly both types of processes occur in nature and we must be concerned with not only the differences in chemical equilibrium of isotopic substances but also the differences in chemical properties related to the kinetics of chemical reactions. In the latter case we are concerned not only with the partition ratios for the reactants but also with the mechanism of the reaction. J. Bigeleisen (2) has attempted to calculate ratios of specific rate constants for isotopic molecules.

It is known that sulphates are reduced to hydrogen sulphide in the muds at the bottom of lakes and seas. Some of this hydrogen sulphide escapes into the atmosphere where it is oxidized by oxygen to free sulphur and sulphuric acid, and some reacts with iron silicates to produce pyrite or mascarite. Pyrite sulphur can be converted to hydrogen sulphide by acids or changed to sulphur dioxide by heat and oxidizing conditions (volcanoes). This hydrogen sulphide and sulphur dioxide can react together at volcano vents to produce free sulphur. In each of the above reactions, fractionation can occur over long periods of time. As the sulphur is recycled among the different compounds, then it is possible to build up considerable differences in the isotopic abundances. Thus by means of the natural "sulphur cycle" (see below) where the sulphur atoms in sulphates, hydrogen sulphide, free sulphur, pyrite, sulphur dioxide, organic sulphur, etc., are interchanged, it is possible to produce the variations in S^{34} that have been reported.

In view of the many variables in naturally occurring processes, it is important that each step in the sulphur cycle be studied under controlled laboratory conditions to determine the isotope fractionation that occurs under different conditions. In this connection, experiments are under way to investigate the reduction of sulphate to hydrogen sulphide by anerobic sulphur bacteria, the oxidation of sulphides to sulphates by aerobic sulphur bacteria, and finally the synthesis of organic sulphur from sulphates in plant photosynthesis.

There are a number of chemical exchange reactions involving the isotopes of sulphur for which equilibrium constants could be measured to check some of

SULPHUR CYCLE

$SO_4^=$ Anaerobic bacteria S^0
                    ―――――――――――――→
                    Oxidation or bacteria

Anaerobic bacteria
Aerobic bacteria
and oxidation

Oxidation

Reduction

Photosynthesis

H_2S SO_2

Decomposition
Photosynthesis

Iron silicates
Acids and weathering

Heat

Organic
S   Sulphides

the theoretical calculations summarized in Table III. The isotope exchange between H_2S and $S^=$ in solution has already been investigated in this laboratory by Mr. A. Szabo (unpublished work). According to his results the equilibrium constant for the reaction

$$H_2S^{32}{}_g + HS^{34-}{}_{sol'n} \rightleftharpoons H_2S^{34}{}_g + HS^{32-}{}_{sol'n} \qquad (a)$$
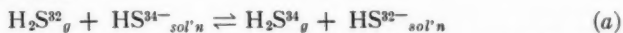
is 1.006 at 25°C. Unfortunately we do not have partition function ratios for HS^- and cannot calculate this equilibrium constant. However, the equilibrium constant for the reaction

$$H_2S^{32}{}_g + S^{34=}{}_{sol'n} \rightleftharpoons H_2S^{34}{}_g + S^{32=}{}_{sol'n} \qquad (b)$$
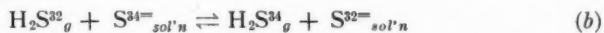
can be obtained from Table III and is 1.013 at 25°C. It is reasonable to assume that the $(K - 1)$ for Reaction (a) will be about $\frac{1}{2}$ of $(K - 1)$ for Reaction (b). Thus the agreement between theory and experiment is fairly good.

The sulphur exchange between H_2S and CS_2, which is analogous to the much studied exchange of oxygen between carbon dioxide and water, would be of

interest. According to Table III the calculated equilibrium constant for the reaction

$$H_2S^{34} + \tfrac{1}{2}CS_2^{32} \rightleftharpoons H_2S^{32} + \tfrac{1}{2}CS_2^{34} \qquad\qquad (c)$$

is 1.006 at 25°C., whereas for the corresponding reaction

$$H_2O^{18} + \tfrac{1}{2}CO_2^{16} \rightleftharpoons H_2O^{16} + \tfrac{1}{2}CO_2^{18}$$
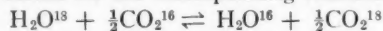
K is 1.044. Although the enrichment factor is less in the exchange S^{34} they are both in the same direction, the heavy isotope being favored in the carbon compound.

Although Douglas, Cooley, and Yost (3) state that CS_2 and H_2S do not exchange in benzene solution, it is felt that an exchange might take place in carbon disulphide solution. This exchange could proceed through the reaction

$$H_2S + CS_2 = H_2CS_3$$
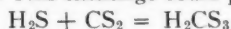
Edwards and Nesbett (4) report that the sulphur isotopes exchange between $S^=_{sol'n}$ and CS_2 through the intermediate $CS_3^=$. The equilibrium constant for the exchange

$$S^{34=} + \tfrac{1}{2}CS_2^{32} \rightleftharpoons S^{32=} + \tfrac{1}{2}CS_2^{34}$$
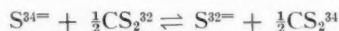
would be 1.019 at 25°C. as calculated from Table III.

Recently Macnamara and Thode (10) reported that the isotopic content of nine different meteoritic samples of sulphur was constant within the limits of the measurements. Further, the constant value obtained falls in the middle of the range of isotopic abundances for terrestrial sources (see Fig. 1). These facts seem to indicate that earth sulphur and meteoritic sulphur are of the same origin, but that terrestrial sulphur has been subjected to chemical fractionation in biological and geological processes.

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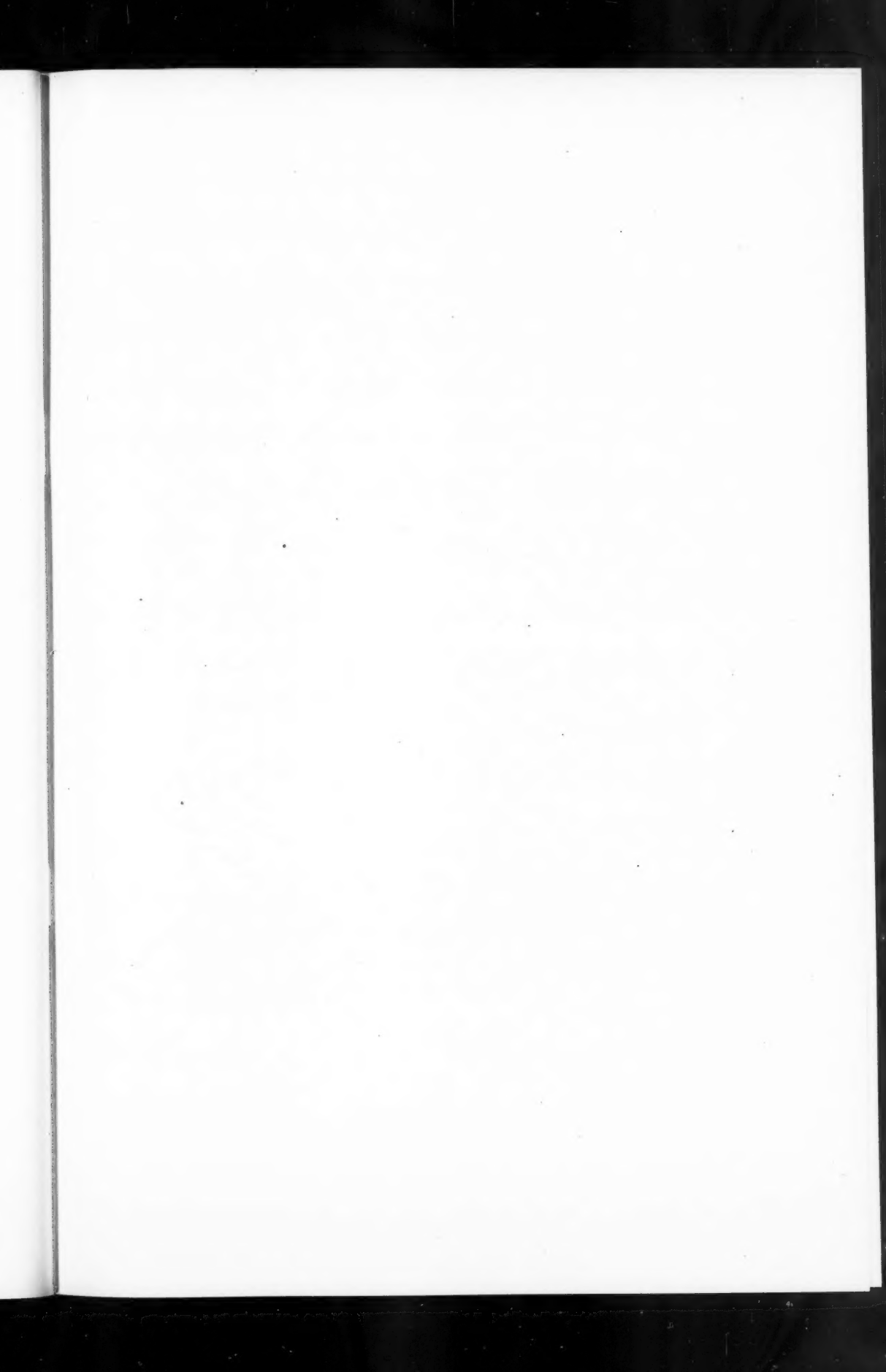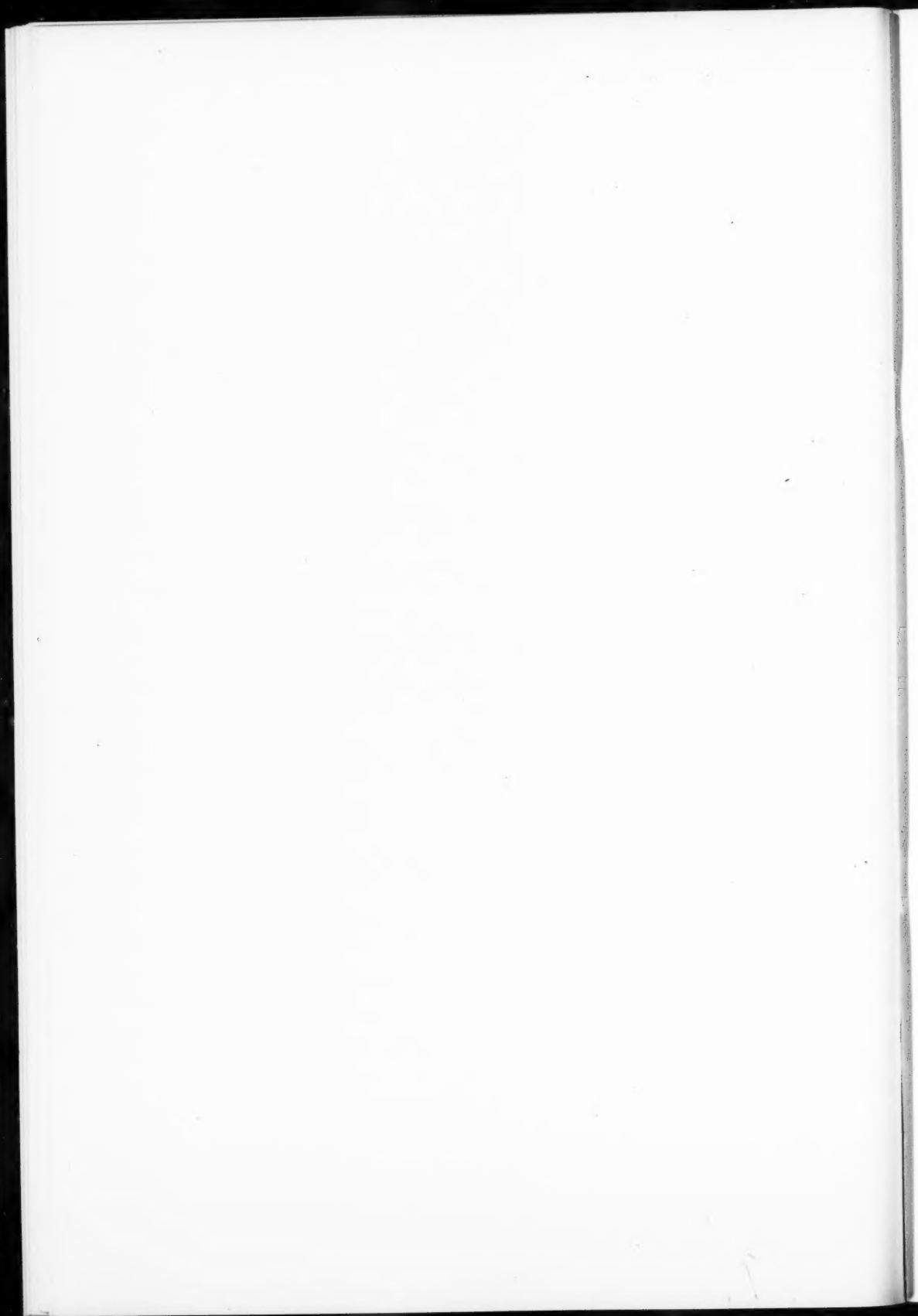
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